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(54) Title: 2-SUBSTITUTED ESTRADIOL DERIVATIVE FOR INHIBITING SUPEROXID DISMUTASE

(57) Abstract: There is provided use of a compound in the manufacture o a medicament to inhibit superoxide dismutase (SOD) or for use in the therapy of a condition or disease associated with SOD wherein the compound is of Formula (I) wherein: X is a ring having at least 4 atoms in the ring; K is a hydrocarbyl group; R^1 is a halogen group of the formula $-L^1-Z-R^{1'}$, wherein L^1 is an optional linker group, Z is O or S and $R^{1'}$ is a hydrocarbyl group or H; with the proviso that the compound is other than 2-methoxy-17β-oestradiol, 2-methoxyoestrone and 2-hydroxyestradiol.

SUBSTITUTED ESTRADIOL DERIVATIVE FOR INHIBITING SUPEROXID DISMUTASE

FIELD OF INVENTION

The present invention relates to a use.

In particular the present invention relates to a use of a compound for the manufacture of a medicament to inhibit superoxide dismutase (SOD). In other aspects the present invention relates to a compound and to a pharmaceutical composition comprising the compound. The present invention also relates to the use of the compound or composition in therapy applications.

BACKGROUND TO THE INVENTION

Estrogens undergo serial hydroxylation, methylation and conjugation reactions and recent evidence demonstrates that at least some of the products of these reactions possess unique biological activities independent of the classical oestrogen receptor (ER). 2-Methoxyestradiol (2-MeOE2) is a hydroxylation and methylation derivative of 17β-estradiol and has received considerable interest as an endogenous growth inhibitory and cytotoxic agent with potential use for cancer therapy.

2-MeOE2 inhibits the growth and/or promotes apoptosis of many malignant cell types, including ER positive and negative human breast cancer and leukaemic cell lines and primary chronic lymphocytic leukaemia cells. Oral administration of 2-MeOE2 inhibits the in vivo growth of transplanted human tumours in immunodeficient mice. In addition to its direct effects on the proliferation and survival of cancer cells, 2-MeOE2 also possesses significant anti-angiogenic activity.

We have generated a series of natural and synthetic oestrone and oestradiol derivatives to further examine the biological effects of this class of molecules and to identify compounds with enhanced activity. Of particular note, we showed that the 3-O-sulfamoylated derivatives of 2-methoxyestrone and 2-ethylestrone (2-methoxyestrone-3-O-sulfamate and 2-ethylestrone-3-O-sulfamate, respectively) were significantly more effective in inhibiting the growth and survival of breast cancer cells relative to their parental oestrones and 2-MeOE2 [Purohit et al 1999; MacCarthy-Morrogh et al 2000].

2-MeOE2 binds to the colchicine binding site on tubulin, and inhibits the polymerisation of tubulin dimers to give microtubules. 2-MeOE2 arrests cells in mitosis and induction of apoptosis is associated with phosphorylation of the anti-apoptotic BCL-2 protein. This is consistent with tubulin as a major target for 2-MeOE2 since other structurally diverse anti-tubulin agents, such as vincristine and paclitaxel have similar effects in cells. The enhanced activity of the 3-O-sulfamoylated oestrones was associated with more potent inhibition of tubulin polymerisation, enhanced mitotic arrest and apoptosis, and an increase in BCL-2 phosphorylation relative to parental compounds and 2-MeOE2.

More recently, 2-MeOE2 has been shown to inhibit the activity of both CuZn and Mn superoxide dismutases (SOD). SOD catalyse the breakdown of superoxide radical and are essential enzymes in protecting cells from reactive oxygen species (ROS). Cancer cells are thought to be particularly sensitive to inhibition of SOD, because of their enhanced production of superoxide ions and their reduced SOD expression. Inhibition of SOD activity and subsequent increase in ROS has been suggested to play a major role in induction of apoptosis by 2-MeOE2. In support of this idea Huang et al., showed that in addition to 2-MeOE2, 2-MeOE1 and 2-hydroxyestradiol, but not oestradiol or oestrone, also inhibited SOD and induced apoptosis. However, both of these compounds would also be expected to inhibit tubulin polymerisation to some extent and therefore this small series of compounds would not differentiate between these two modes of action. Therefore, it is not clear what are the key structural features of estrogens required for optimal activity.

The present invention seeks to provide novel compounds suitable for the inhibition of SOD as well as other therapeutic applications.

SUMMARY ASPECTS OF THE PRESENT INVENTION

The present invention is based on the surprising finding that certain compounds could be used as effective superoxide dismutase (SOD) inhibitors and/or as agents that can influence apoptosis.

In one aspect, the present invention is based on the surprising finding that certain substituted cyclic compounds could be used as effective superoxide dismutase inhibitors

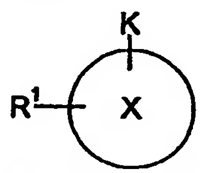
and/or as modulators of apoptosis.

The substituted cyclic compounds comprise at least one group containing O or S that is a substituent of a ring system. The ring system compounds comprise at least one ring component. That ring component comprises at least 4 atoms in the ring. Typically, those 4 atoms will be carbon atoms. Thus, typically, that ring component will be a hydrocarbyl group.

The compounds of the present invention may comprise other substituents. These other substituents may, for example, further increase the activity of the compounds of the present invention and/or increase stability (*ex vivo* and/or *in vivo*).

DETAILED ASPECTS OF THE PRESENT INVENTION

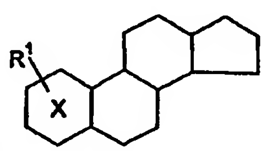
According to one aspect of the present invention, there is provided use of a compound in the manufacture of a medicament to inhibit superoxide dismutase (SOD) or for use in the therapy of a condition or disease associated with SOD wherein the compound is of Formula I



Formula I

wherein: X is a ring having at least 4 atoms in the ring; K is a hydrocarbyl group; R^1 is a halogen or a group of the formula $-L^1-Z-R^{1'}$, wherein L^1 is an optional linker group, Z is O or S and $R^{1'}$ is a hydrocarbyl group or H; with the proviso that the compound is other than 2-methoxy-17β-oestradiol, 2-methoxyoestrone and 2-hydroxyestradiol.

According to one aspect of the present invention, there is provided a compound having Formula IX



Formula IX

wherein R^1 is a group of the formula $-L^1-S-R^{1'}$, wherein L^1 is an optional linker group, and $R^{1'}$ is a hydrocarbyl group or H.

According to one aspect of the present invention, there is provided a compound according to the present invention for use in medicine.

According to one aspect of the present invention, there is provided a pharmaceutical composition comprising the compound according to the present invention optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

According to one aspect of the present invention, there is provided the use of a compound according to the present invention in the manufacture of a medicament for use in the therapy of a condition or disease associated with SOD and/or apoptosis.

According to one aspect of the present invention, there is provided the use of a compound according to the present invention in the manufacture of a medicament for use in the therapy of a condition or disease associated with adverse SOD and/or apoptosis.

According to one aspect of the present invention, there is provided a method comprising (a) performing a superoxide dismutase assay with one or more candidate compounds of Formula I; (b) determining whether one or more of said candidate compounds is/are capable of modulating SOD activity and/or apoptosis; and (c) selecting one or more of said candidate compounds that is/are capable of modulating SOD activity and/or apoptosis.

According to one aspect of the present invention, there is provided a method comprising (a) performing a superoxide dismutase assay with one or more candidate compounds having of Formula I; (b) determining whether one or more of said candidate compounds is/are capable of inhibiting SOD activity; and (c) selecting one or more of said candidate compounds that is/are capable of inhibiting SOD activity and/or apoptosis.

In any one of the methods of the present invention, one or more additional steps may be present. For example, the method may also include the step of modifying the identified candidate compound (such as by chemical and/or enzymatic techniques) and the optional additional step of testing that modified compound for SOD inhibition effects (which may be to see if the effect is greater or different). By way of further example, the method may also include the step of determining the structure (such as by use of

crystallographic techniques) of the identified candidate compound and then performing computer modelling studies – such as to further increase its SOD inhibitory action. Thus, the present invention also encompasses a computer having a dataset (such as the crystallographic co-ordinates) for said identified candidate compound. The present invention also encompasses that identified candidate compound when presented on a computer screen for the analysis thereof – such as protein binding studies.

According to one aspect of the present invention, there is provided a compound identified by the method of the present invention.

The present invention also encompasses the novel compounds of the present invention (such as those presented herein), as well as processes for making same (such as the processes presented herein) as well as novel intermediates (such as those presented herein) for use in those processes.

SOME ADVANTAGES

One key advantage of the present invention is that the compounds of the present invention can act as SOD inhibitors.

The compounds of the present invention may be used for the in the therapy of a condition or disease associated with SOD, adverse SOD levels and/or apoptosis. These therapies included therapies to induce apoptosis and to overcome resistance to apoptosis. This is advantageous in condition where cells should have died but have not. Yet further the inhibitors of the present invention may be utilised to counter defects in other enzymes involved in the regulation of reactive oxygen species. Thus in a further aspect the present invention provides a therapy or use of a compound of the present invention for the regulation of a reactive oxygen metabolic pathway.

Another advantage of the compounds of the present invention is that they may be potent *in vivo*.

Some of the compounds of the present invention may be non-oestrogenic compounds. Here, the term "non-oestrogenic" means exhibiting no or substantially no oestrogenic activity.

Another advantage is that some of the compounds may not be capable of being metabolised to compounds which display or induce hormonal activity.

Some of the compounds of the present invention are also advantageous in that they may be orally active.

Some of the compounds of the present invention may useful for the treatment of cancer, such as breast cancer, as well as (or in the alternative) non-malignant conditions, such as the prevention of auto-immune diseases, particularly when pharmaceuticals may need to be administered from an early age.

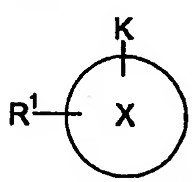
Thus, some of the compounds of the present invention are also believed to have therapeutic uses other than for the treatment of cancer, such as the treatment of autoimmune diseases.

The compounds of the present invention may also be useful as an inducer of apoptosis.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

PREFERABLE ASPECTS

As discussed above the present invention provides use of a compound in the manufacture of a medicament to inhibit superoxide dismutase (SOD) or for use in the therapy of a condition or disease associated with SOD wherein the compound is of Formula I



Formula I

wherein: X is a ring having at least 4 atoms in the ring; K is a hydrocarbyl group; R^1 is a halogen or a group of the formula $-L^1-Z-R^{1'}$, wherein L^1 is an optional linker group, Z is O or S and $R^{1'}$ is a hydrocarbyl group or H; with the proviso that the compound is other than

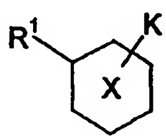
2-methoxy-17β-oestradiol, 2-methoxyoestrone and 2-hydroxyestradiol.

It will be understood that when Z is S depending on the oxidation state of the sulphur, the S may have other groups attached thereto. For example the S may be divalent, tri- or tetracoordinated. Examples of such groups which may be attached to the S include =O.

In one aspect the compound is other than 2-methoxyoestradiol (i.e. 2-methoxy-17β-oestradiol and 2-methoxy-17α-oestradiol), 2-methoxyoestrone and 2-hydroxyestradiol.

Preferably the ring X is a 6 membered ring.

Preferably the compound of the present invention Formula II



Formula II

Group K need not be a cyclic structure. In this regard, group K may be a linear structure that may have the ability to conform to a ring like structure when in *in vivo*.

In a preferred aspect, group K is cyclic - so as to form the cyclic group K. In this aspect X is a ring and K is a cyclic group. Thus the compound is a polycyclic compound.

Cyclic group K need not necessarily be fused to ring X. In this regard, they may be separated by a suitable spacer group – which may be a hydrocarbyl group.

In a preferred aspect, cyclic group K is fused to ring X.

Group K may be a polycyclic group, which need not be a fused polycycle.

Preferably X in combination with K is a polycyclic ring structure. Thus, in a preferred aspect, group K and ring X make up a polycyclic compound. As indicated, here the term "polycyclic" includes fused and non-fused ring structures including combinations thereof.

At least one of the cyclic groups K and X may be a heterocyclic group (a heterocycle) or a

non-heterocyclic group.

At least one of the cyclic groups K and X may be a saturated ring structure or an unsaturated ring structure (such as an aryl group).

Preferably, at least one of the cyclic groups is an aryl ring.

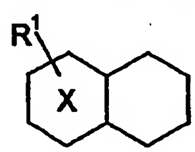
If the cyclic group is polycyclic some or all of the ring components of the compound may be fused together or joined *via* one or more suitable spacer groups.

The polycyclic compound may comprise a number of fused rings. In this aspect the fused rings may comprise any combination of different size rings, such as 3 six-membered rings (6,6,6), a six-membered ring, a seven-membered ring and a six-membered ring (6,7,6), a six-membered ring and two eight-membered rings (6,8,8) etc.

In one aspect the present invention relates to compounds wherein the polycyclic compounds are other than (6,6,7) rings. In a further aspect, the present invention relates to compounds wherein the polycyclic compounds only contain rings having other than 7 members.

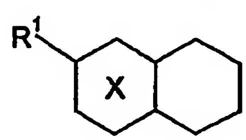
The polycyclic compound can comprise at least two ring components, or at least three ring components, or at least four ring components.

Preferably the compound of the present invention is of Formula III



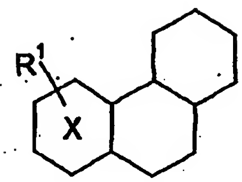
Formula III

Preferably the compound of the present invention is of Formula IV



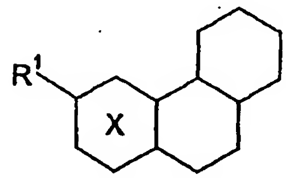
Formula IV

Preferably the compound of the present invention is of Formula V



Formula V

Preferably the compound of the present invention is of Formula VI



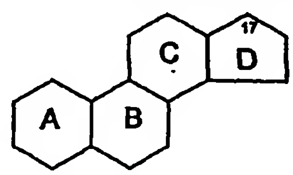
Formula VI

Preferably, the polycyclic compound comprises four ring components.

Preferred polycyclic compounds have a steroidal ring component, or bio-isosteres thereof.

In a preferred aspect X in combination with K is a steroidal ring structure - that is to say a cyclopentanophenanthrene skeleton, or bio-isosteres thereof.

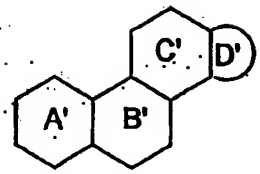
As it is well known in the art, a classical steroidal ring structure has the generic formula of:



In the above formula, the rings have been labelled in the conventional manner.

An example of a bio-isostere is when any one or more of rings A, B, C and D is a heterocyclic ring and/or when any one or more of rings A, B, C and D has been substituted and/or when any one or more of rings A, B, C and D has been modified; but wherein the bio-isostere in the absence of the sulphamate group has steroidal properties.

In this regard, the structure of a preferred polycyclic structure can be presented as:



wherein each ring A', B', C' and D' independently represents a heterocyclic ring or a non-heterocyclic ring, which rings may be independently substituted or unsubstituted, saturated or unsaturated.

By way of example, any one or more of rings A', B', C' and D' may be independently substituted with suitable groups - such as an alkyl group, an aryl group, a hydroxy group, a halo group, a hydrocarbyl group, an oxyhydrocarbyl group etc.

An example of D' is a five or six membered non-heterocyclic ring having at least one substituent.

In one preferred embodiment, the ring D' is substituted with a ethinyl group.

If any one of rings A', B', C' and D' is a heterocyclic ring, then preferably that heterocyclic ring comprises a combination of C atoms and at least one N atom and/or at least one O atom. Other heterocyclic atoms may be present in the ring.

Examples of suitable, preferred steroidal nuclei rings A'-D' of the compounds of the present invention include rings A-D of dehydroepiandrosterone and oestrogens including oestrone.

Preferred steroidal nuclei rings A'-D' of the compounds of the present invention include rings A-D of:

<u>oestrones and substituted oestrones, *viz*:</u>

oestrone

2-OH-oestrone

4-OH-oestrone

6α-OH-oestrone

7α-OH-oestrone
16α-OH-oestrone
16β-OH-oestrone
2-MeO-oestrone
17-deoxyoestrone

<u>oestradiols and substituted oestradiols, *viz*:</u>
4-OH-17β-oestradiol
6α-OH-17β-oestradiol
7α-OH-17β-oestradiol
4-OH-17α-oestradiol
6α-OH-17α-oestradiol
7α-OH-17α-oestradiol
16α-OH-17α-oestradiol
16α-OH-17β-oestradiol
16β-OH-17α-oestradiol
16β-OH-17β-oestradiol
17α-oestradiol
17β-oestradiol
17α-ethinyl-17β-oestradiol
17β-ethinyl-17α-oestradiol
17-deoxyoestradiol

<u>oestriols and substituted oestriols, *viz*:</u>
oestriol
4-OH-oestriol
6α-OH-oestriol
7α-OH-oestriol
17-deoxyoestriol

<u>dehydroepiandrosterones and substituted dehydroepiandrosterones, *viz*:</u>
dehydroepiandrosterones
6α-OH-dehydroepiandrosterone

7α-OH-dehydroepiandrosterone
16α-OH-dehydroepiandrosterone
16β-OH-dehydroepiandrosterone
androstenediol

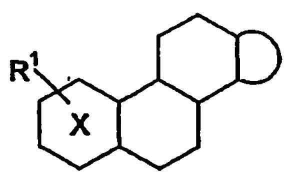
In general terms the ring system A'B'C'D' may contain a variety of non-interfering substituents. In particular, the ring system A'B'C'D' may contain one or more hydroxy, alkyl especially lower (C_1-C_6) alkyl, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl and other pentyl isomers, and n-hexyl and other hexyl isomers, alkoxy especially lower (C_1-C_6) alkoxy, e.g. methoxy, ethoxy, propoxy etc., alkinyl, e.g. ethinyl, or halogen, e.g. fluoro substituents.

In a preferred aspect X in combination with K mimics a steroidal structure. The term "mimic" as used herein means having a similar or different structure but having a similar functional effect. In other words, group K and ring X together may be a bio-isostere of the rings of a steroid, or an active part thereof.

In a preferred aspect, group K and ring X together may be a bio-isostere of the rings of oestrone, or a part thereof.

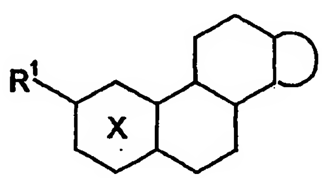
Preferably the compound of the present invention is of Formula VII

Formula VII

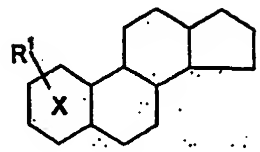


Preferably the compound of the present invention is of Formula VIII

Formula VIII

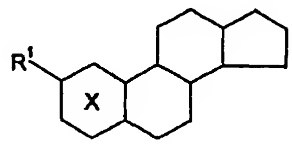


Preferably the compound of the present invention is of Formula IX



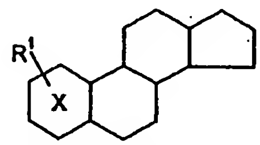
Formula IX

Preferably the compound of the present invention is of Formula X



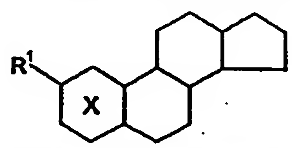
Formula X

The present invention provides novel compounds having Formula IX



Formula IX

and preferably having Formula X



Formula X

wherein R^1 is a group of the formula $-L^1-S-R^{1'}$, wherein L^1 is an optional linker group, and $R^{1'}$ is a hydrocarbyl group or H.

Preferably for the compound of the present invention R^1 is a group of the formula $-S-R^{1'}$.

Preferably for the compound of the present invention $R^{1'}$ is an alkyl group.

Preferably for the compound of the present invention $R^{1'}$ is a C_1-C_{10} alkyl group.

Preferably for the compound of the present invention $R^{1'}$ is a C_1-C_6 alkyl group.

Preferably for the compound of the present invention $R^{1'}$ is a C_1-C_3 alkyl group.

Preferably for the compound of the present invention $R^{1'}$ is $-CH_3$ or $-CH_2CH_3$.

Preferably for the compound of the present invention R^1 is selected from $-SCH_3$, –

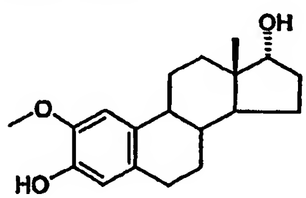
SCH_2CH_3, $-S(O)CH_3$, and $-S(O)(O)CH_3$

In a further aspect the present invention also provides the compound described above for use in medicine.

In a further aspect the present invention also provides a pharmaceutical composition comprising the compound described above optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

In a further aspect the present invention also provides use of the compound described above in the manufacture of a medicament for the inhibition of SOD or for use in the therapy of a condition or disease associated with SOD.

Further novel compounds are provided by the present invention. These compound include compounds 4, 9, 17, 22, 23 and 29. The structures of these compounds are given below



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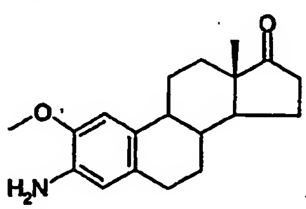

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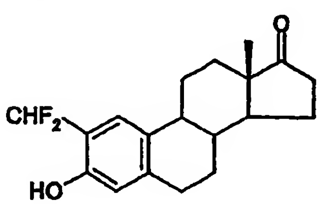

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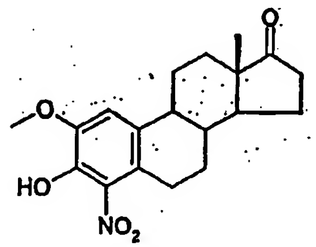

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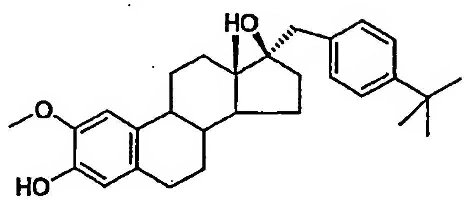

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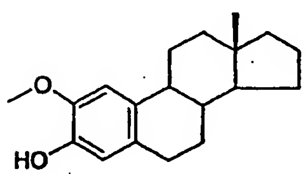

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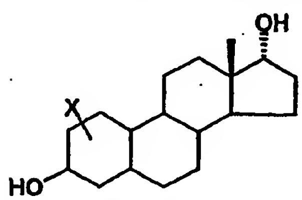
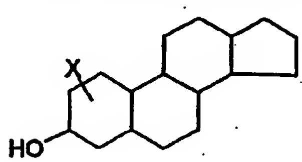
Thus in aspect of the invention there is provided

- a compound having the formula



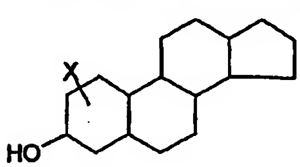
wherein X is an oxyhydrocarbyl group as defined herein

- a compound of the formula



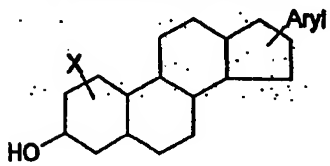
wherein X is a halohydrocarbyl group as defined below

- a compound of the formula



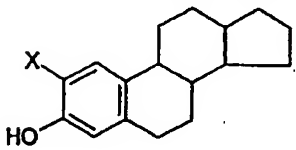
wherein X is a nitro group

- a compound of the formula



wherein X is an oxyhydrocarbyl group as defined herein and Aryl is an aryl group

- a compound of the formula



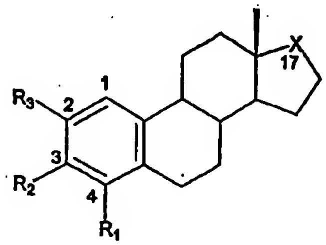
wherein X is an oxyhydrocarbyl group as defined herein

The term "halohydrocarbyl" group as used herein means a group comprising at least C, H and a halogen (i.e. Cl, Br, I and/or F) and may optionally comprise one or more other suitable substituents. Examples of such substituents may include oxy-, alkoxy-, nitro-, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the oxyhydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the oxyhydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur and nitrogen.

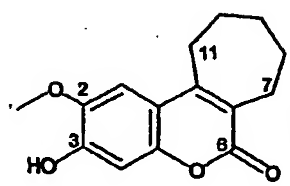
We have surprisingly found that compounds containing a non-steroidal ring structure may act as described herein. Compounds for use in the present invention may not contain or be based on a steroid nucleus. In this regard, the polycyclic compound may contain or be based on a non-steroidal ring system - such as diethylstilboestrol, stilboestrol, coumarins, flavonoids, combrestatin and other ring systems. Other suitable non-steroidal compounds for use in or as the composition of the present invention may be found in US-A-5567831. In preferred aspects of the invention

- when R1 is a methoxy group or hydroxy group, X in combination with K is a non-steroidal ring structure; and/or
- the compound for use in the present invention is non-steroidal; and/or
- X in combination with K is a non-steroidal ring structure

In a highly preferred aspect the compound for use in the present invention is a compound selected from compounds of the formula



R_1	R_2	R_3	X	
H	OH	MeS	CH(β-OH)	1
H	OH	EtO	CH(β-OH)	2
H	OH	MeO	CH(α-OH)	4
H	OH	EtS	CH(β-OH)	6
H	OH	EtO	C=O	7
H	OH	MeS	C=O	8
H	NH_2	MeO	C=O	9
H	OH	EtS	C=O	10
H	OH	F	C=O	11
H	OH	Cl	C=O	12
H	OH	MeO	C(β-OH)(α-Bn)	13
H	OH	H_2C=$CHCH_2$	C=O	15
H	OH	I	C=O	16
H	OH	CHF_2	C=O	17
H	OH	MeSO	C=O	18
H	OH	$MeSO_2$	C=O	19
H	OH	NO_2	C=O	20
H	OH	Et	C=O	21
NO_2	OH	MeO	C=O	22
H	OH	MeO	C[β-OH][α-(4-^{t}Bu)Bn]	23
H	OSO_2NH_2	H	C=O	24
H	OSO_2NH_2	MeO	C=O	25
H	OSO_2NH_2	Et	C=O	26



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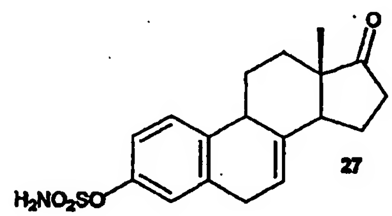

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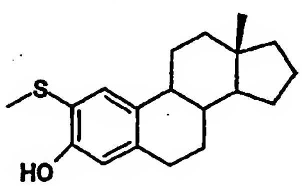

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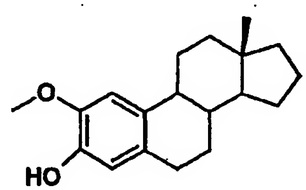

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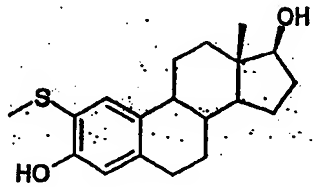
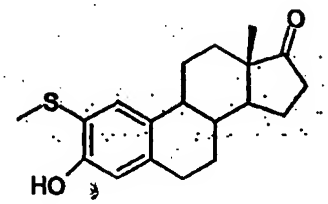
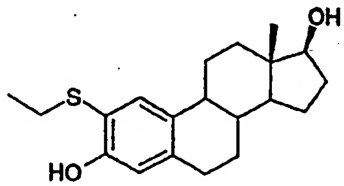
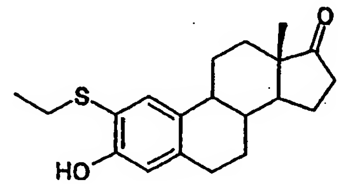
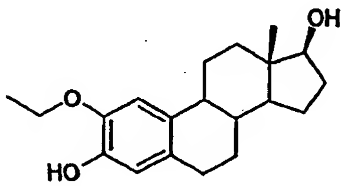
Highly preferred compounds are compounds of the formulae



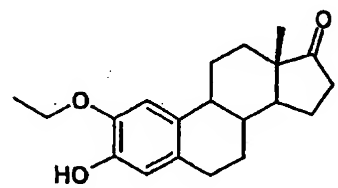




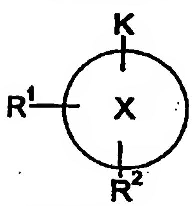




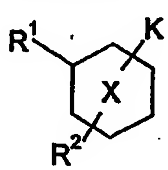
and



In a preferred aspect ring X is substituted with group R2. Thus in this aspect the compound for use in the present invention is



preferably



R^2 may be any suitable substituent. Examples of R^2 include -OH and amine or amide groups such as NH_2.

Preferably the group K and the ring X together will contain, inclusive of all substituents, a maximum of about 50 carbon atoms, more usually no more than about 30 to 40 carbon atoms.

In a preferred aspect the compounds of the present invention or for use therein are not substituted with a sulphamate group. As would be understood by one skilled in the art a sulphamate group is includes an ester of sulphamic acid, or an ester of an N-substituted derivative of sulphamic acid, or a salt thereof.

For some applications, preferably the compounds have no, or a minimal, oestrogenic effect.

For some applications, preferably the compounds have an oestrogenic effect.

For some applications, preferably the compounds have a reversible action.

For some applications, preferably the compounds have an irreversible action.

In one embodiment, the compounds of the present invention are useful for the treatment of breast cancer.

The present invention also covers novel intermediates that are useful to prepare the compounds of the present invention.

SUPEROXIDE DISMUTASE

As discussed by Huang *et al.* Nature, Vol 407, 21 September 2000, 390-395 superoxide dismutases (SOD) are essential enzymes that eliminate superoxide radical (O^{2-}) and thus protect cells from damage induced by free radicals. The active O^{2-} production and low SOD activity in cancer cells may render the malignant cells highly dependent on SOD for survival and sensitive to inhibition of SOD. Inhibition of SOD causes accumulation of cellular O^{2-} and leads to free-radical-mediated damage to mito-chondrial membranes, the release of cytochrome *c* from mito-chondria and apoptosis of the cancer cells. Targeting SOD is a promising approach to the selective killing of cancer cells, and that mechanism-based combinations of SOD inhibitors with free-radical-producing agents may have clinical applications.

SOD INHIBITOR

In accordance with the present invention, the compound of the present invention is capable of acting as an SOD inhibitor.

Here, the term "inhibitor" as used herein with respect to the compound of the present invention means a compound that can inhibit SOD activity – such as reduce and/or eliminate and/or mask and/or prevent the action of SOD. The SOD inhibitor may act as an antagonist.

The ability of compounds to inhibit superoxide dismutase activity can be assessed using the SOD assay of Protocol 1.

For some applications, preferably the compound of the present invention has at least about a 100 fold selectivity to a desired target (e.g. SOD), preferably at least about a 150 fold selectivity to the desired target, preferably at least about a 200 fold selectivity to the desired target, preferably at least about a 250 fold selectivity to the desired target, preferably at least about a 300 fold selectivity to the desired target, preferably at least about a 350 fold selectivity to the desired target.

It is to be noted that the compound of the present invention may have other beneficial properties in addition to or in the alternative to its ability to inhibit SOD activity.

GROUP R^1

Group R^1 of the compound for use in the present invention is a halogen or a group of the formula $-L^1$-Z-$R^{1'}$. L^1 is an optional linker group, Z is O or S and $R^{1'}$ is a hydrocarbyl group or H;

If present L^1 may be a hydrocarbyl group.

Preferably L^1 is selected from C_1-C_{10} hydrocarbyl, C_1-C_5 hydrocarbyl or C_1-C_3 hydrocarbyl.

Preferably L^1 is selected from hydrocarbon groups, preferably C_1-C_{10} hydrocarbon, C_1-C_5

hydrocarbon or C_1-C_3 hydrocarbon.

Preferably L^1 is selected from alkyl groups, C_1-C_{10} alkyl, C_1-C_6 alkyl or C_1-C_3 alkyl.

The hydrocarbyl/hydrocarbon/alkyl of L^1 may be straight chain or branched and/or may be saturated or unsaturated.

In one aspect L^1 may be linked to the remainder of R^1.

In one aspect R^1 is a halogen. The halogen may be F, Cl, Br or I. In this aspect preferably the halogen is selected from F and Cl.

In one aspect R^1 is a group of the formula $-L^1$-Z-$R^{1'}$. L^1 is an optional linker group. When L^1 is present R^1 is a group of the formula $-L^1$-Z-$R^{1'}$. When L^1 is not present in one aspect R^1 is a group of the formula $-$Z-$R^{1'}$

Group Z may be O or S. For the avoidance of doubt O represent oxygen and S represents sulphur. In one aspect Z is O. In one aspect Z is S.

$R^{1'}$ is a hydrocarbyl group or H.

Preferably $R^{1'}$ is selected from C_1-C_{10} hydrocarbyl, C_1-C_6 hydrocarbyl or C_1-C_3 hydrocarbyl.

Preferably $R^{1'}$ is selected from hydrocarbon groups, C_1-C_{10} hydrocarbon, C_1-C_6 hydrocarbon or C_1-C_3 hydrocarbon.

In a preferred aspect $R^{1'}$ is an alkyl group.

Preferably $R^{1'}$ is a C_1-C_{10} alkyl group, more preferably a C_1-C_6 alkyl group, more preferably a C_1-C_3 alkyl group, more preferably $-CH_3$ or $-CH_2CH_3$.

In further preferred aspects $R^{1'}$ is a C_2-C_{10} alkyl group, preferably a C_2-C_6 alkyl group, preferably a C_2 or C_3 alkyl group.

The hydrocarbyl/hydrocarbon/alkyl of $R^{1'}$ may be straight chain or branched and/or may be saturated or unsaturated.

In a highly preferred aspect R^1 is selected from $-SCH_3$, OCH_3, $-SCH_2CH_3$, $-OCH_2CH_3$, $-F$, and $-Cl$.

In a preferred aspect when Z is O, $R^{1'}$ is a C_2-C_{10} alkyl group and when Z is S, $R^{1'}$ is a hydrocarbyl group or H

HYDROCARBYL

The term "hydrocarbyl group" as used herein means a group comprising at least C and H and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo, alkoxy, nitro, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the hydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked via a suitable element or group. Thus, the hydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur, nitrogen and oxygen. A non-limiting example of a hydrocarbyl group is an acyl group.

A typical hydrocarbyl group is a hydrocarbon group. Here the term "hydrocarbon" means any one of an alkyl group, an alkenyl group, an alkynyl group, which groups may be linear, branched or cyclic, or an aryl group. The term hydrocarbon also includes those groups but wherein they have been optionally substituted. If the hydrocarbon is a branched structure having substituent(s) thereon, then the substitution may be on either the hydrocarbon backbone or on the branch; alternatively the substitutions may be on the hydrocarbon backbone and on the branch.

HYDROCARBYLSULPHANYL

When Z is S the group of the formula $-L^1$-Z-$R^{1'}$ may be referred to or is analogous to a hydrocarbylsulphanyl group.

The term "hydrocarbylsulphanyl" means a group that comprises at least hydrocarbyl group (as herein defined) and sulphur. That sulphur group may be optionally oxidised.

OXYHYDROCARBYL

When Z is O the group of the formula $-L^1\text{-}Z\text{-}R^{1'}$ may be referred to or is analogous to an oxyhydrocarbyl group.

The term "oxyhydrocarbyl" group as used herein means a group comprising at least C, H and O and may optionally comprise one or more other suitable substituents. Examples of such substituents may include halo-, alkoxy-, nitro-, an alkyl group, a cyclic group etc. In addition to the possibility of the substituents being a cyclic group, a combination of substituents may form a cyclic group. If the oxyhydrocarbyl group comprises more than one C then those carbons need not necessarily be linked to each other. For example, at least two of the carbons may be linked *via* a suitable element or group. Thus, the oxyhydrocarbyl group may contain hetero atoms. Suitable hetero atoms will be apparent to those skilled in the art and include, for instance, sulphur and nitrogen.

In one embodiment of the present invention, the oxyhydrocarbyl group is a oxyhydrocarbon group.

Here the term "oxyhydrocarbon" means any one of an alkoxy group, an oxyalkenyl group, an oxyalkynyl group, which groups may be linear, branched or cyclic, or an oxyaryl group. The term oxyhydrocarbon also includes those groups but wherein they have been optionally substituted. If the oxyhydrocarbon is a branched structure having substituent(s) thereon, then the substitution may be on either the hydrocarbon backbone or on the branch; alternatively the substitutions may be on the hydrocarbon backbone and on the branch.

OTHER SUBSTITUENTS

The compound of the present invention may have substituents other than R^1. By way of example, these other substituents may be one or more of: one or more halo groups, one or more O groups, one or more hydroxy groups, one or more amino groups, one or more sulphur containing group(s), one or more hydrocarbyl group(s) – such as an

oxyhydrocarbyl group.

SUPEROXIDE DISMUTASE ASSAY (PROTOCOL 1)

SOD activity was measured using a spectrophotometric assay (Ukeda 1997). In this reaction, 3'-{1-[(phenylamino)-carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT) is converted to a colourimetric product by the action of superoxide ions (produced by xanthine oxidase (XO) and xanthine). Production of superoxide ions is inhibited by SOD resulting in decreased product. The ability of test compounds to inhibit SOD is measured by their ability to restore production of the coloured product. Reactions were performed in a total volume of 0.1 ml of buffer A (42 mM Na_2CO_3 (pH 9.4), 0.1 mM xanthine (Sigma), 0.1 mM EDTA, 0.026 mM XTT (Sigma)).

The reaction was initiated by addition of 3.3 µl of xanthine oxidase (Sigma, 64 mU/ml) and the amount of CuZnSOD (Sigma) added was titrated to give 80-90% inhibition of XO activity. The reaction was incubated at 24 °C for 30 minutes and products determined by measuring absorobance at 470 nm. All determinations were made in duplicate/triplicate.

BIOTECHNOLOGICAL ASSAYS FOR DETERMINING SOD ACTIVITY (PROTOCOL 2)

The ability of compounds to inhibit superoxide dismutase activity can also be assessed using amino acid sequences or nucleotide sequences encoding SOD, or active fragments, derivatives, homologues or variants thereof in, for example, high-through put screens.

Any one or more of appropriate targets - such as an amino acid sequence and/or nucleotide sequence - may be used for identifying an agent capable of modulating SOD in any of a variety of drug screening techniques. The target employed in such a test may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The abolition of target activity or the formation of binding complexes between the target and the agent being tested may be measured.

The assay of the present invention may be a screen, whereby a number of agents are tested. In one aspect, the assay method of the present invention is a high through put screen.

Techniques for drug screening may be based on the method described in Geysen, European Patent Application 84/03564, published on September 13, 1984. In summary, large numbers of different small peptide test compounds are synthesised on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with a suitable target or fragment thereof and washed. Bound entities are then detected - such as by appropriately adapting methods well known in the art. A purified target can also be coated directly onto plates for use in a drug screening techniques. Alternatively, non-neutralising antibodies can be used to capture the peptide and immobilise it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralising antibodies capable of binding a target specifically compete with a test compound for binding to a target.

Another technique for screening provides for high throughput screening (HTS) of agents having suitable binding affinity to the substances and is based upon the method described in detail in WO 84/03564.

It is expected that the assay methods of the present invention will be suitable for both small and large-scale screening of test compounds as well as in quantitative assays.

In one preferred aspect, the present invention relates to a method of identifying agents that selectively modulate SOD, which compounds have the formula (Ia).

<u>REPORTERS</u>

A wide variety of reporters may be used in the assay methods (as well as screens) of the present invention with preferred reporters providing conveniently detectable signals (e.g. by spectroscopy). By way of example, a reporter gene may encode an enzyme which catalyses a reaction which alters light absorption properties.

Other protocols include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilising monoclonal antibodies reactive to two non-interfering epitopes may even be used. These and other assays are described, among other places, in Hampton R et al (1990, Serological Methods, A Laboratory Manual, APS Press, St Paul MN) and Maddox DE et al (1983, J Exp Med 15 8:121 1).

Examples of reporter molecules include but are not limited to (β-galactosidase, invertase, green fluorescent protein, luciferase, chloramphenicol, acetyltransferase, (-glucuronidase, exo-glucanase and glucoamylase. Alternatively, radiolabelled or fluorescent tag-labelled nucleotides can be incorporated into nascent transcripts which are then identified when bound to oligonucleotide probes.

By way of further examples, a number of companies such as Pharmacia Biotech (Piscataway, NJ), Promega (Madison, WI), and US Biochemical Corp (Cleveland, OH) supply commercial kits and protocols for assay procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US-A-3817837; US-A-3850752; US-A-3939350; US-A-3996345; US-A-4277437; US-A-4275149 and US-A-4366241.

HOST CELLS

The term "host cell" - in relation to the present invention includes any cell that could comprise the target for the agent of the present invention.

Thus, a further embodiment of the present invention provides host cells transformed or transfected with a polynucleotide that is or expresses the target of the present invention. Preferably said polynucleotide is carried in a vector for the replication and expression of polynucleotides that are to be the target or are to express the target. The cells will be chosen to be compatible with the said vector and may for example be prokaryotic (for example bacterial), fungal, yeast or plant cells.

The gram negative bacterium *E. coli* is widely used as a host for heterologous gene expression. However, large amounts of heterologous protein tend to accumulate inside

the cell. Subsequent purification of the desired protein from the bulk of *E.coli* intracellular proteins can sometimes be difficult.

In contrast to *E.coli*, bacteria from the genus *Bacillus* are very suitable as heterologous hosts because of their capability to secrete proteins into the culture medium. Other bacteria suitable as hosts are those from the genera Streptomyces and Pseudomonas.

Depending on the nature of the polynucleotide encoding the polypeptide of the present invention, and/or the desirability for further processing of the expressed protein, eukaryotic hosts such as yeasts or other fungi may be preferred. In general, yeast cells are preferred over fungal cells because they are easier to manipulate. However, some proteins are either poorly secreted from the yeast cell, or in some cases are not processed properly (e.g. hyperglycosylation in yeast). In these instances, a different fungal host organism should be selected.

Examples of suitable expression hosts within the scope of the present invention are fungi such as Aspergillus species (such as those described in EP-A-0184438 and EP-A-0284603) and Trichoderma species; bacteria such as Bacillus species (such as those described in EP-A-0134048 and EP-A-0253455), Streptomyces species and Pseudomonas species; and yeasts such as Kluyveromyces species (such as those described in EP-A-0096430 and EP-A-0301670) and Saccharomyces species. By way of example, typical expression hosts may be selected from *Aspergillus niger*, *Aspergillus niger var. tubigenis*, *Aspergillus niger var. awamori*, *Aspergillus aculeatis*, *Aspergillus nidulans*, *Aspergillus orvzae*, *Trichoderma reesei*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Kluyveromyces lactis* and *Saccharomyces cerevisiae*.

The use of suitable host cells - such as yeast, fungal and plant host cells - may provide for post-translational modifications (e.g. myristoylation, glycosylation, truncation, lapidation and tyrosine, serine or threonine phosphorylation) as may be needed to confer optimal biological activity on recombinant expression products of the present invention.

<u>ORGANISM</u>

The term "organism" in relation to the present invention includes any organism that could comprise the target according to the present invention and/or products obtained

therefrom. Examples of organisms may include a fungus, yeast or a plant.

The term "transgenic organism" in relation to the present invention includes any organism that comprises the target according to the present invention and/or products obtained.

TRANSFORMATION OF HOST CELLS/HOST ORGANISMS

As indicated earlier, the host organism can be a prokaryotic or a eukaryotic organism. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press) and Ausubel *et al.*, Current Protocols in Molecular Biology (1995), John Wiley & Sons, Inc.

If a prokaryotic host is used then the nucleotide sequence may need to be suitably modified before transformation - such as by removal of introns.

In another embodiment the transgenic organism can be a yeast. In this regard, yeast have also been widely used as a vehicle for heterologous gene expression. The species Saccharomyces cerevisiae has a long history of industrial use, including its use for heterologous gene expression. Expression of heterologous genes in *Saccharomyces cerevisiae* has been reviewed by Goodey *et al* (1987, Yeast Biotechnology, D R Berry *et al*, eds, pp 401-429, Allen and Unwin, London) and by King *et al* (1989, Molecular and Cell Biology of Yeasts, E F Walton and G T Yarronton, eds, pp 107-133, Blackie, Glasgow).

For several reasons *Saccharomyces cerevisiae* is well suited for heterologous gene expression. First, it is non-pathogenic to humans and it is incapable of producing certain endotoxins. Second, it has a long history of safe use following centuries of commercial exploitation for various purposes. This has led to wide public acceptability. Third, the extensive commercial use and research devoted to the organism has resulted in a wealth of knowledge about the genetics and physiology as well as large-scale fermentation characteristics of Saccharomyces cerevisiae.

A review of the principles of heterologous gene expression in *Saccharomyces cerevisiae* and secretion of gene products is given by E Hinchcliffe E Kenny (1993, "Yeast as a vehicle for the expression of heterologous genes", Yeasts, Vol 5, Anthony H Rose and J Stuart Harrison, eds, 2nd edition, Academic Press Ltd.).

Several types of yeast vectors are available, including integrative vectors, which require recombination with the host genome for their maintenance, and autonomously replicating plasmid vectors.

In order to prepare the transgenic Saccharomyces, expression constructs are prepared by inserting the nucleotide sequence into a construct designed for expression in yeast. Several types of constructs used for heterologous expression have been developed. The constructs contain a promoter active in yeast fused to the nucleotide sequence, usually a promoter of yeast origin, such as the GAL1 promoter, is used. Usually a signal sequence of yeast origin, such as the sequence encoding the SUC2 signal peptide, is used. A terminator active in yeast ends the expression system.

For the transformation of yeast several transformation protocols have been developed. For example, a transgenic Saccharomyces according to the present invention can be prepared by following the teachings of Hinnen et al (1978, Proceedings of the National Academy of Sciences of the USA 75, 1929); Beggs, J D (1978, Nature, London, 275, 104); and Ito, H et al (1983, J Bacteriology 153, 163-168).

The transformed yeast cells are selected using various selective markers. Among the markers used for transformation are a number of auxotrophic markers such as LEU2, HIS4 and TRP1, and dominant antibiotic resistance markers such as aminoglycoside antibiotic markers, e.g. G418.

Another host organism is a plant. The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material. Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech

March/April 1994 17-27). Further teachings on plant transformation may be found in EP-A-0449375.

Thus, the present invention also provides a method of transforming a host cell with a nucleotide sequence that is to be the target or is to express the target. Host cells transformed with the nucleotide sequence may be cultured under conditions suitable for the expression of the encoded protein. The protein produced by a recombinant cell may be displayed on the surface of the cell. If desired, and as will be understood by those of skill in the art, expression vectors containing coding sequences can be designed with signal sequences which direct secretion of the coding sequences through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join the coding sequence to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll DJ et al (1993) DNA Cell Biol 12:441-53).

VARIANTS/HOMOLOGUES/DERIVATIVES

In addition to the specific amino acid sequences and nucleotide sequences mentioned herein, the present invention also encompasses the use of variants, homologue and derivatives thereof. Here, the term "homology" can be equated with "identity".

In the present context, an homologous sequence is taken to include an amino acid sequence which may be at least 75, 85 or 90% identical, preferably at least 95 or 98% identical. Although homology can also be considered in terms of similarity (i.e. amino acid residues having similar chemical properties/functions), in the context of the present invention it is preferred to express homology in terms of sequence identity.

Homology comparisons can be conducted by eye, or more usually, with the aid of readily available sequence comparison programs. These commercially available computer programs can calculate % homology between two or more sequences.

% homology may be calculated over contiguous sequences, i.e. one sequence is aligned with the other sequence and each amino acid in one sequence is directly compared with the corresponding amino acid in the other sequence, one residue at a time. This is called an "ungapped" alignment. Typically, such ungapped alignments are performed only over a relatively short number of residues.

Although this is a very simple and consistent method, it fails to take into consideration that, for example, in an otherwise identical pair of sequences, one insertion or deletion will cause the following amino acid residues to be put out of alignment, thus potentially resulting in a large reduction in % homology when a global alignment is performed. Consequently, most sequence comparison methods are designed to produce optimal alignments that take into consideration possible insertions and deletions without penalising unduly the overall homology score. This is achieved by inserting "gaps" in the sequence alignment to try to maximise local homology.

However, these more complex methods assign "gap penalties" to each gap that occurs in the alignment so that, for the same number of identical amino acids, a sequence alignment with as few gaps as possible - reflecting higher relatedness between the two compared sequences - will achieve a higher score than one with many gaps. "Affine gap costs" are typically used that charge a relatively high cost for the existence of a gap and a smaller penalty for each subsequent residue in the gap. This is the most commonly used gap scoring system. High gap penalties will of course produce optimised alignments with fewer gaps. Most alignment programs allow the gap penalties to be modified. However, it is preferred to use the default values when using such software for sequence comparisons. For example when using the GCG Wisconsin Bestfit package (see below) the default gap penalty for amino acid sequences is -12 for a gap and -4 for each extension.

Calculation of maximum % homology therefore firstly requires the production of an optimal alignment, taking into consideration gap penalties. A suitable computer program for carrying out such an alignment is the GCG Wisconsin Bestfit package (University of Wisconsin, U.S.A.; Devereux et al., 1984, Nucleic Acids Research 12:387). Examples of other software than can perform sequence comparisons include, but are not limited to, the BLAST package (see Ausubel et al., 1999 ibid – Chapter 18), FASTA (Atschul et al., 1990, J. Mol. Biol., 403-410) and the GENEWORKS suite of comparison tools. Both BLAST and FASTA are available for offline and online searching (see Ausubel et al., 1999 ibid, pages 7-58 to 7-60). However it is preferred to use the GCG Bestfit program.

A further useful reference is that found in FEMS Microbiol Lett 1999 May 15;174(2):247-50 (and a published erratum appears in FEMS Microbiol Lett 1999 Aug 1;177(1):187-8).

Although the final % homology can be measured in terms of identity, the alignment process itself is typically not based on an all-or-nothing pair comparison. Instead, a scaled similarity score matrix is generally used that assigns scores to each pairwise comparison based on chemical similarity or evolutionary distance. An example of such a matrix commonly used is the BLOSUM62 matrix - the default matrix for the BLAST suite of programs. GCG Wisconsin programs generally use either the public default values or a custom symbol comparison table if supplied (see user manual for further details). It is preferred to use the public default values for the GCG package, or in the case of other software, the default matrix, such as BLOSUM62.

Once the software has produced an optimal alignment, it is possible to calculate % homology, preferably % sequence identity. The software typically does this as part of the sequence comparison and generates a numerical result.

The sequences may also have deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent substance. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the secondary binding activity of the substance is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine, asparagine, glutamine, serine, threonine, phenylalanine, and tyrosine.

Conservative substitutions may be made, for example according to the Table below. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

EXPRESSION VECTORS

The nucleotide sequence for use as the target or for expressing the target can be incorporated into a recombinant replicable vector. The vector may be used to replicate and express the nucleotide sequence in and/or from a compatible host cell. Expression may be controlled using control sequences which include promoters/enhancers and other expression regulation signals. Prokaryotic promoters and promoters functional in eukaryotic cells may be used. Tissue specific or stimuli specific promoters may be used. Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

The protein produced by a host recombinant cell by expression of the nucleotide sequence may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. The coding sequences can be designed with signal sequences which direct secretion of the substance coding sequences through a particular prokaryotic or eukaryotic cell membrane.

FUSION PROTEINS

The target amino acid sequence may be produced as a fusion protein, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), 6xHis, GAL4 (DNA binding and/or transcriptional activation domains) and (-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences. Preferably the fusion protein will not hinder the activity of the target.

The fusion protein may comprise an antigen or an antigenic determinant fused to the substance of the present invention. In this embodiment, the fusion protein may be a non-naturally occurring fusion protein comprising a substance which may act as an adjuvant in the sense of providing a generalised stimulation of the immune system. The antigen or antigenic determinant may be attached to either the amino or carboxy terminus of the substance.

In another embodiment of the invention, the amino acid sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for agents capable of affecting the substance activity, it may be useful to encode a chimeric substance expressing a heterologous epitope that is recognised by a commercially available antibody.

THERAPY

The compounds of the present invention may be used as therapeutic agents – i.e. in therapy applications.

The term "therapy" includes curative effects, alleviation effects, and prophylactic effects.

The therapy may be on humans or animals, preferably female animals.

PHARMACEUTICAL COMPOSITIONS

In one aspect, the present invention provides a pharmaceutical composition, which comprises a compound according to the present invention and optionally a pharmaceutically acceptable carrier, diluent or excipient (including combinations thereof).

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s).

Preservatives, stabilisers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents

may be also used.

There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestable solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

Where the agent is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion, solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

COMBINATION PHARMACEUTICAL

The compound of the present invention may be used in combination with one or more other active agents, such as one or more other pharmaceutically active agents.

By way of example, the compounds of the present invention may be used in combination with other SOD inhibitors and/or free radical producing agents and/or other inhibitors such

as an aromatase inhibitor (such as for example, 4-hydroxyandrostenedione (4-OHA)) and/or steroids – such as the naturally occurring neurosteroids dehydroepiandrosterone sulphate (DHEAS) and pregnenolone sulphate (PS) and/or other structurally similar organic compounds.

COMBINATION THERAPIES

The inhibitors of the present invention may also be combined with other therapies.

For example the compound may be administered in combination with radiotherapy. Radiotherapy is believed to work by generating reactive oxygen. Combination of the present compounds with radiotherapy will provide improved results, particularly in tumours resistant to radiotherapy alone.

OTHER ASPECTS

The present invention may be used in other non-medicinal applications. For example the present compound mat be utilised as an anti-microbial. Macrophages use reactive oxygen to kill micro-organisms. Some micro-organisms express a SOD enzyme. The SOD is often required for pathogenicity. Thus by provision of a SOD inhibiting compound the present invention may provide more effective macrophage action.

ADMINISTRATION

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The dosages below are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited.

The compositions of the present invention may be administered by direct injection. The composition may be formulated for parenteral, rectal, vaginal, mucosal, intramuscular, intravenous, subcutaneous, intraocular or transdermal administration. Depending upon the need, the agent may be administered at a dose of from 0.01 to 30 mg/kg body weight, such as from 0.1 to 10 mg/kg, more preferably from 0.1 to 1 mg/kg body weight.

By way of further example, the agents of the present invention may be administered in accordance with a regimen of 1 to 4 times per day, preferably once or twice per day. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

Aside from the typical modes of delivery – indicated above – the term "administered" also includes delivery by techniques such as lipid mediated transfection, liposomes, immunoliposomes, lipofectin, cationic facial amphiphiles (CFAs) and combinations thereof. The routes for such delivery mechanisms include but are not limited to mucosal, nasal, oral, parenteral, gastrointestinal, topical, or sublingual routes.

The term "administered" includes but is not limited to delivery by a mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestable solution; a parenteral route where delivery is by an injectable form, such as, for example, an intravenous, intramuscular or subcutaneous route.

Thus, for pharmaceutical administration, the SOD inhibitors of the present invention can be formulated in any suitable manner utilising conventional pharmaceutical formulating techniques and pharmaceutical carriers, adjuvants, excipients, diluents etc. and usually for parenteral administration. Approximate effective dose rates may be in the range from 1 to 1000 mg/day, such as from 10 to 900 mg/day or even from 100 to 800 mg/day depending on the individual activities of the compounds in question and for a patient of average (70Kg) bodyweight. More usual dosage rates for the preferred and more active compounds will be in the range 200 to 800 mg/day, more preferably, 200 to 500 mg/day, most preferably from 200 to 250 mg/day. They may be given in single dose regimes, split dose regimes and/or in multiple dose regimes lasting over several days. For oral administration they may be formulated in tablets, capsules, solution or suspension containing from 100 to 500 mg of compound per unit dose. Alternatively and preferably the compounds will be formulated for parenteral administration in a suitable parenterally administrable carrier and providing single daily dosage rates in the range 200 to 800 mg, preferably 200 to 500, more preferably 200 to 250 mg. Such effective daily doses will, however, vary depending on inherent activity of the active ingredient and on the

bodyweight of the patient, such variations being within the skill and judgement of the physician.

CANCER

As indicated, the compounds of the present invention may be useful in the treatment of cancer.

Cancer remains a major cause of mortality in most Western countries. Cancer therapies developed so far have included blocking the action or synthesis of hormones to inhibit the growth of hormone-dependent tumours. However, more aggressive chemotherapy is currently employed for the treatment of hormone-independent tumours.

Hence, the development of a pharmaceutical for anti-cancer treatment of hormone dependent and/or hormone independent tumours, yet lacking some or all of the side-effects associated with chemotherapy, would represent a major therapeutic advance.

We believe that the compounds of the present invention provides a means for the treatment of cancers and, especially, breast cancer.

In addition or in the alternative the compound of the present invention may be useful in the blocking the growth of cancers including leukaemias and solid tumours such as breast, endometrium, prostate, ovary and pancreatic tumours.

NEURODEGENERATIVE DISEASES

We believe that some of the compounds of the present invention may be useful in the treatment of neurodenerative diseases, and similar conditions.

By way of example, it is believed that SOD inhibitors may be useful in the enhancing the memory function of patients suffering from illnesses such as amnesia, head injuries, Alzheimer's disease, epileptic dementia, presenile dementia, post traumatic dementia, senile dementia, vascular dementia and post-stroke dementia or individuals otherwise seeking memory enhancement.

INFLAMATORY CONDITIONS

We believe that some of the compounds of the present invention may be useful in treating inflammatory conditions – such as conditions associated with any one or more of: autoimmunity, including for example, rheumatoid arthritis, type I and II diabetes, systemic lupus erythematosus, multiple sclerosis, myasthenia gravis, thyroiditis, vasculitis, ulcerative colitis and Crohn's disease, skin disorders e.g. psoriasis and contact dermatitis; graft versus host disease; eczema; asthma and organ rejection following transplantation.

By way of example, it is believed that SOD inhibitors may prevent the normal physiological effect of DHEA or related steroids on immune and/or inflammatory responses.

The compounds of the present invention may be useful in the manufacture of a medicament for revealing an endogenous glucocorticoid-like effect.

OTHER THERAPIES

It is also to be understood that the compound/composition of the present invention may have other important medical implications.

For example, the compound or composition of the present invention may be useful in the treatment of the disorders listed in WO-A-99/52890 – *viz*:

In addition, or in the alternative, the compound or composition of the present invention may be useful in the treatment of the disorders listed in WO-A-98/05635. For ease of reference, part of that list is now provided: cancer, inflammation or inflammatory disease, dermatological disorders, fever, cardiovascular effects, haemorrhage, coagulation and acute phase response, cachexia, anorexia, acute infection, HIV infection, shock states, graft-versus-host reactions, autoimmune disease, reperfusion injury, meningitis, migraine and aspirin-dependent anti-thrombosis; tumour growth, invasion and spread, angiogenesis, metastases, malignant, ascites and malignant pleural effusion; cerebral ischaemia, ischaemic heart disease, osteoarthritis, rheumatoid arthritis, osteoporosis, asthma, multiple sclerosis, neurodegeneration, Alzheimer's disease, atherosclerosis, stroke, vasculitis, Crohn's disease and ulcerative colitis; periodontitis, gingivitis;

psoriasis, atopic dermatitis, chronic ulcers, epidermolysis bullosa; corneal ulceration, retinopathy and surgical wound healing; rhinitis, allergic conjunctivitis, eczema, anaphylaxis; restenosis, congestive heart failure, endometriosis, atherosclerosis or endosclerosis.

In addition, or in the alternative, the compound or composition of the present invention may be useful in the treatment of disorders listed in WO-A-98/07859. For ease of reference, part of that list is now provided: cytokine and cell proliferation/differentiation activity; immunosuppressant or immunostimulant activity (e.g. for treating immune deficiency, including infection with human immune deficiency virus; regulation of lymphocyte growth; treating cancer and many autoimmune diseases, and to prevent transplant rejection or induce tumour immunity); regulation of haematopoiesis, e.g. treatment of myeloid or lymphoid diseases; promoting growth of bone, cartilage, tendon, ligament and nerve tissue, e.g. for healing wounds, treatment of burns, ulcers and periodontal disease and neurodegeneration; inhibition or activation of follicle-stimulating hormone (modulation of fertility); chemotactic/chemokinetic activity (e.g. for mobilising specific cell types to sites of injury or infection); haemostatic and thrombolytic activity (e.g. for treating haemophilia and stroke); antiinflammatory activity (for treating e.g. septic shock or Crohn's disease); as antimicrobials; modulators of e.g. metabolism or behaviour; as analgesics; treating specific deficiency disorders; in treatment of e.g. psoriasis, in human or veterinary medicine.

In addition, or in the alternative, the composition of the present invention may be useful in the treatment of disorders listed in WO-A-98/09985. For ease of reference, part of that list is now provided: macrophage inhibitory and/or T cell inhibitory activity and thus, anti-inflammatory activity; anti-immune activity, i.e. inhibitory effects against a cellular and/or humoral immune response, including a response not associated with inflammation; inhibit the ability of macrophages and T cells to adhere to extracellular matrix components and fibronectin, as well as up-regulated fas receptor expression in T cells; inhibit unwanted immune reaction and inflammation including arthritis, including rheumatoid arthritis, inflammation associated with hypersensitivity, allergic reactions, asthma, systemic lupus erythematosus, collagen diseases and other autoimmune diseases, inflammation associated with atherosclerosis, arteriosclerosis, atherosclerotic heart disease, reperfusion injury, cardiac arrest, myocardial infarction, vascular inflammatory disorders, respiratory distress syndrome or other cardiopulmonary

diseases, inflammation associated with peptic ulcer, ulcerative colitis and other diseases of the gastrointestinal tract, hepatic fibrosis, liver cirrhosis or other hepatic diseases, thyroiditis or other glandular diseases, glomerulonephritis or other renal and urologic diseases, otitis or other oto-rhino-laryngological diseases, dermatitis or other dermal diseases, periodontal diseases or other dental diseases, orchitis or epididimo-orchitis, infertility, orchidal trauma or other immune-related testicular diseases, placental dysfunction, placental insufficiency, habitual abortion, eclampsia, pre-eclampsia and other immune and/or inflammatory-related gynaecological diseases, posterior uveitis, intermediate uveitis, anterior uveitis, conjunctivitis, chorioretinitis, uveoretinitis, optic neuritis, intraocular inflammation, e.g. retinitis or cystoid macular oedema, sympathetic ophthalmia, scleritis, retinitis pigmentosa, immune and inflammatory components of degenerative fondus disease, inflammatory components of ocular trauma, ocular inflammation caused by infection, proliferative vitreo-retinopathies, acute ischaemic optic neuropathy, excessive scarring, e.g. following glaucoma filtration operation, immune and/or inflammation reaction against ocular implants and other immune and inflammatory-related ophthalmic diseases, inflammation associated with autoimmune diseases or conditions or disorders where, both in the central nervous system (CNS) or in any other organ, immune and/or inflammation suppression would be beneficial, Parkinson's disease, complication and/or side effects from treatment of Parkinson's disease, AIDS-related dementia complex HIV-related encephalopathy, Devic's disease, Sydenham chorea, Alzheimer's disease and other degenerative diseases, conditions or disorders of the CNS, inflammatory components of stokes, post-polio syndrome, immune and inflammatory components of psychiatric disorders, myelitis, encephalitis, subacute sclerosing pan-encephalitis, encephalomyelitis, acute neuropathy, subacute neuropathy, chronic neuropathy, Guillaim-Barre syndrome, Sydenham chora, myasthenia gravis, pseudo-tumour cerebri, Down's Syndrome, Huntington's disease, amyotrophic lateral sclerosis, inflammatory components of CNS compression or CNS trauma or infections of the CNS, inflammatory components of muscular atrophies and dystrophies, and immune and inflammatory related diseases, conditions or disorders of the central and peripheral nervous systems, post-traumatic inflammation, septic shock, infectious diseases, inflammatory complications or side effects of surgery, bone marrow transplantation or other transplantation complications and/or side effects, inflammatory and/or immune complications and side effects of gene therapy, e.g. due to infection with a viral carrier, or inflammation associated with AIDS, to suppress or inhibit a humoral and/or cellular immune response, to treat or ameliorate monocyte or leukocyte proliferative diseases,

e.g. leukaemia, by reducing the amount of monocytes or lymphocytes, for the prevention and/or treatment of graft rejection in cases of transplantation of natural or artificial cells, tissue and organs such as cornea, bone marrow, organs, lenses, pacemakers, natural or artificial skin tissue.

SUMMARY

In summation, the present invention provides a method for the manufacture of a medicament for inhibiting SOD and/or modulating apoptosis and to novel compounds for use in the same and pharmaceutical compositions containing them.

EXAMPLES

The present invention will now be described only by way of example. However, it is to be understood that the examples also present preferred compounds of the present invention, as well as preferred routes for making same and useful intermediates in the preparation of same.

Materials and Methods

Chemical Synthesis

The structures of the compounds tested are shown in Figure 1.

2-Substituted oestrones and oestradiols were synthesised from oestrone by either electrophilic substitution (X = F,Cl,Br, I) or by ortho-lithiation of appropriately protected oestrone and quenching with the appropriate electrophile (X = OR, SR etc.) followed by standard transformations to the target compound.

2-Haloestrone (**11**, **12** and **16**) were prepared as described by Page Bulman PC, Hussain F, Maggs JL, Morgan P and Park BK. (1990) Efficient regioselective A-ring functionalisation of oestrogens. *Tetrahedron* 46: 2059-2068

2-Methoxy-17α-estradiol, 4 was prepared by inversion of the 3-O-benzyl derivative of 2-Methoxy-17β-estradiol under Mitsunobu reaction conditions followed by base hydrolysis

and hydrogenolysis.

2-Methoxy-3-aminoestrone, **9** was prepared from the 3-(2'-phenyl-4'-quinazolinyloxy) derivative of 2-methoxyestrone in a similar manner to the preparation of 3-aminoestrone as described by Woo LWL, Lightowler, Purohit A, Reed MJ and Potter BVL (1996) Heteroatom-substituted analogues of the active site-directed inhibitor estra-1,3,5(10)-trien-17-one-3-sulphamate inhibit oestrone sulfatase by a different mechanism. *J. Steroid Biochem. Molec. Biol.* 57: 79-88.

The coumarin derivative, **14** was prepared by reacting 4-methoxy-1,3-benzenediol and methyl 2-oxo-1-cycloheptanecarboxylate under Pechmann conditions.

2-Allylestrone, **15** was prepared by a Claisen rearrangement of oestrone allyl ether as described by Patton TL (1962) Estrogens. IV. The synthesis of 2- and 4-alkylestrones. *J. Org. Chem.* 27: 910-914.

2-Difluoromethylestrone, **17** was prepared by fluorination of a suitably bis-protected 2-formylestrone with [bis(2-methoxyethyl)amino]sulphur trifluoride (Deoxo-Fluor reagent - Lal GS, Pez PP, Pesaresi RJ, Prozonic FM and Cheng HS (1999) [Bis(2-methoxyethyl)amino]sulphur trifluoride: A new broad-spectrum deoxofluorinating agent with enhanced thermal stability. *J. Org. Chem.* 64: 7048-7054) followed by deprotection.

2-Methylsulfoxyestrone, **18** and 2-methylsulfonylestrone, **19** were prepared by oxidation of 2-methylsulfanylestrone with 1.7 eq., at $0^{o}C$ and 3.5 eq., at room temperature of 3-chloroperoxybenzoic acid respectively.

2-Nitroestrone, **20** was prepared by nitration of oestrone as described by Tomson AJ and Horwitz JP (1959) Some 2- and 4-substituted oestrone 3-methyl ethers. *J. Org. Chem.* 24: 2056-2058.

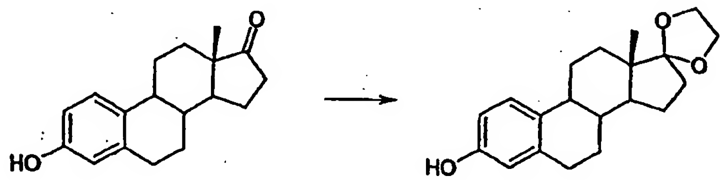
2-Methoxy-4-nitroestrone, **22** was prepared by nitration of 2-methoxyestrone with nitronium tetrafluoroborate.

2-Methoxy-17α-benzyl-estradiol, **13** and 2-methoxy-17α-(4-tert-butylbenzyl)oestradiol **23**

were prepared by direct Grignard reaction on 2-methoxyestrone. Sulfamoylation of phenolic compounds with sulfamoyl chloride was carried out as described by Woo *et. al*. Stock solutions were made up at 50 mM in tetrahydrofuran and stored at −20°C.

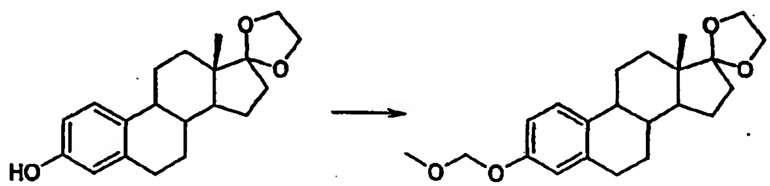
Synthesis of 2-Methylsulfanyl-1,3,5[10]-estratriene-3-ol (8)

17,17-Ethylenedioxy-1,3,5[10]-estratriene-3-ol



A suspension of oestrone (12.5g, 46.2 mmol), toluene (150 ml), ethylene glycol (14 ml) and tosic acid (120 mg) was refluxed for 14h under Dean-Stark conditions. The resultant pale pink transparent solution was poured onto saturated sodium bicarbonate solution (150 ml) and diluted with ethyl acetate (250 ml). The washed organic layer was separated and the aqueous residues extracted with a further alliquot of ethyl acetate (100 ml), the combined organics were washed with water (150 ml) and brine (150 ml), dried and evaporated to yield crude dioxolone (15 g, 103 %) as an off-white crystalline solid m.p. 183-184°C which was used without further purification (NMR shows purity >95%).

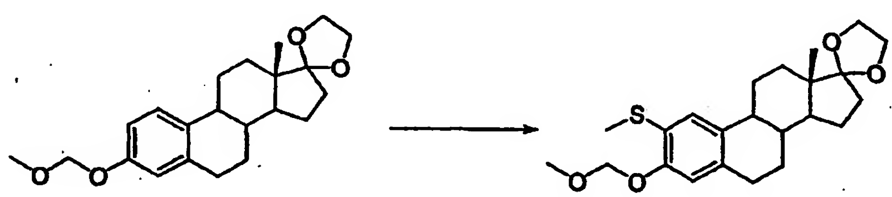
17,17-Ethylenedioxy-3-*O*-methoxymethylene-1,3,5[10]-estratriene



Sodium hydride (1.91 g, 47.7 mmol, 1.5 eq.) was added portion wise to a 0°C solution of the protected oestrone (18.2 g, 58 mmol) in dimethylformamide (250 ml), after H_2 evolution had ceased methyl chloromethyl ether (8.81 ml, 2 eq.) was added in a dropwise

manner and the stirred solution was allowed to come to room temperature overnight. The solution was then poured onto ammonia solution (100 ml, 2M) to destroy any remnant haloether contaminants. Ethyl acetate (500 ml) was added and the aqueous layer removed, the organic layer was then washed with brine (5 x 200 ml) in a portion wise manner, dried and evaporated. The resultant pale yellow oil was purified by column chromatography (4:1 hexane:ethyl acetate, 10 cm diameter column, 20 cm bed depth) to give the product as a colourless oil (18.11g, 88 %) which solidified to give a white crystalline solid m.p. 62-63°C. A further 1.6 g of product was obtained by repurification of mixed column fractions. m.p. 62-63°C, Rf 0.55 (3:1 Hex/EtOAc). δ_H (270 MHz, $CDCl_3$, ref. TMS = 0) 7.21 (1H, d, J 8.6, ArH), 6.82 (1H, d, J 8.6, ArH), 6.77 (1H, app s, ArH), 5.13 (2H, s, OCH_2O), 3.89-3.98 (4H, m, OCH_2CH_2O), 3.46 (3H, s, OCH_3), 2.83 (2H, cm, CH_2), 2.27 (2H, cm, CH_2), 1.25-2.05 (11H, m) and 0.88 (3H, s, CH_3). δ_C 155.0, 138.1, 134.0 (all C), 126.3 (CH), 119.4 (C), 116.2, 113.7 (both CH), 94.5, 65.2, 64.5 (all CH_2), 55.9 (CH_3), 49.3 (CH), 46.1 (C), 43.7, 38.9 (both CH), 34.2, 30.7, 29.7, 26.9, 26.1, 22.3 (all CH_2) and 14.3 (CH_3); MS [EI] 358.2(100 %, M^+); HRMS [EI] 358.214410 $C_{22}H_{30}O_4$ requires 358.21441.

2-Methylsulfanyl-3-*O*-methoxymethylene-17,17-ethylenedioxy-1,3,5[10]-estratriene

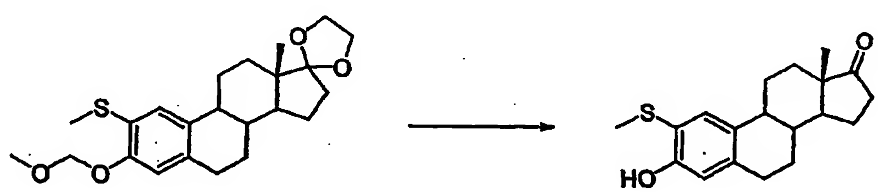


A stirred solution of protected oestrone (20 g, 55.8 mmol) in THF (400 ml) was cooled in a dry ice/acetone bath to –78°C before treating with *sec*-butyl lithium (129ml, 167 mmol, 1.3 M solution in cyclohexane) in a dropwise manner over a period of 1.5h. The anion was maintained at this temperature for a further hour before then quenching with dimethyl disulfide (20 ml, 223 mmol) over a period of five minutes and then allowed to warm to room temperature over a further 2h.At this stage saturated ammonium chloride solution (25 ml) was added, the quenched solution diluted in diethyl ether (250 ml) and the

organic layers were washed with saturated sodium hydrogen carbonate solution (3 x 150 ml), water (150 ml) and finally brine (150 ml) before drying ($MgSO_4$) and evaporating to yield a very pale yellow oil. Purification by gradient elution with hexane/ethyl acetate mixtures (100:0 to 85:15) on silica gel gave a pure fraction of the desired sulphide (14.6 g, 36 mmol, 65%) as a clear colourless oil, a further batch of the sulphide (5.9g, 14.5 mmol) was recovered from the mixed by chromatography to give an overall yield of 20.5g, 91%.

δ_H 7.16 (1H, s, ArH), 6.81 (1H, s, ArH), 5.21 (2H, s, OCH_2O) 3.86-3.96 (4H, m, OCH_2CH_2O), 3.51 (3H, s, OMe), 2.43 (3H, s, SMe), 1.25-2.38 (13H, m) and 0.88 (3H, s, 18-CH_3).

2-Methylsulfanyl-1,3,5[10]-estratriene-3-ol (8)

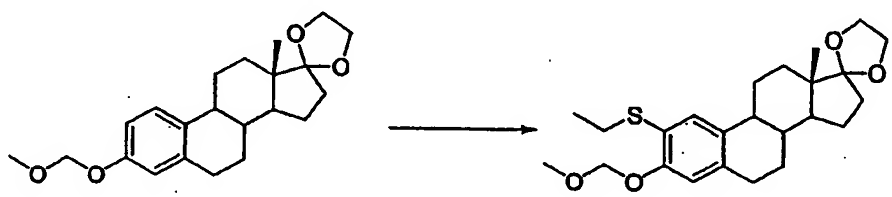


A 4M solution of methanolic HCl was prepared by cautious addition of acetyl chloride (6.1 ml) to ice cold methanol (15.6 ml), after five minutes stirring this solution was poured onto protected oestrone X (1.5g, 3.72 mmol). Sonication was applied to speed the dissolution of the protected oestrone, a pink colour emerged in the reaction mixture after five minutes and, after a further ten minutes, ethyl acetate (100 ml), and then sufficient sodium hydrogen carbonate to neutralise the reaction mixture was added. The organic layer was then washed with water (2 x 50 ml) and brine (100 ml) before drying ($MgSO_4$) and evaporating. Purification by gradient elution with hexane/ethyl acetate mixtures (100:0 to 80:20) on silica gel gave a white crystalline solid mp. 153-5°C which showed: δ_H (400 MHz, $CDCl_3$) 7.40 (1H, s, OH), 6.73 (1H, s, ArH), 6.49 (1H, s, ArH),2.82-2.91 (2H, m,6-CH_2), 2.34-2.56 (2H, m), 2.30 (3H, s, SMe) 1.36-2.26 (11H, m) and 0.91 (3H, s, 18-CH_3); δ_C 226.4 (CO),154.0, 139.8, 132.5(all C), 131.9, 117.9(both CH), 114.5 (C), 50.4(CH), 48.0 (C), 43.9, 38.2 (both CH) 35.9, 31.6, 29.5, 26.5, 26.0, 21.7(all CH_2) 20.3

(SCH_3) and 13.9(CH_3); Calculated C, 72.11, H, 7.64; Found C 71.8 %, H, 7.70 %; MS[FAB$^+$] 316.2 (100%, M$^+$); HRMS[FAB$^+$] 316.149702 calculated 316.14970.

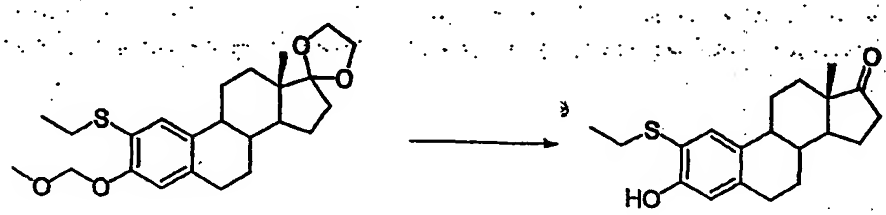
Synthesis of 2-Ethylsulfanyl-1,3,5[10]-estratriene-3-ol (10)

2-Ethylsulfanyl-3-*O*-methoxymethylene-17,17-ethylenedioxy-1,3,5[10]-estratriene



A stirred solution of protected oestrone (5 g, 13.9 mmol) in THF (100 ml) was cooled in a dry ice/acetone bath to –78°C before treating with *sec*-butyl lithium (32.2 ml, 41.8 mmol, 1.3 M solution in cyclohexane) in a dropwise manner over a period of 0.5h. The anion was maintained at this temperature for a further hour and then quenched with diethyl disulfide (9.1 ml, 70 mmol) over a period of five minutes and then allowed to warm to room temperature over a further 2h. At this stage saturated ammonium chloride solution (10 ml) was added, the quenched solution diluted in diethyl ether (100 ml) and the organic layers were washed with saturated sodium hydrogen carbonate solution (3 x 50 ml), water (50 ml) and finally brine (50 ml) before drying ($MgSO_4$) and evaporating to yield a very pale yellow oil. Purification by gradient elution with hexane/ethyl acetate mixtures (100:0 to 85:15) on silica gel gave a pure fraction of the desired sulphide (5.3 g, 12.6 mmol, 97 %) as a clear colourless oil which showed 7.24 (1H, s, ArH), 6.82 (1H, s, ArH), 5.21 (2H, ArOCH_2), 3.86-3.98 (4H, m, OCH_2CH_2O), 3.51 (3H, s, OMe), 2.79-2.94 (4H, m, SCH_2 and 6-CH_2) 1.20-2.36 (16H, m [including 1.30 (3H, t, SCH_2CH_3) and 0.88 (3H, s, 18-CH_3); δ_C 153.3, 136.3, 134.2 (all C), 127.9 (CH), 121.9, 119.3 (both C), 115.0 (CH), 94.8, 65.3, 64.6 (all CH_2) 56.2 (CH3), 49.3 (CH), 46.2 (C), 43.7, 38.9 (both CH), 34.3, 30.7, 29.6, 27.1, 27.0, 26.2, 22.4 (all CH_2), 14.4 and 14.3 (both CH_3)

2-Ethylsulfanyl-1,3,5[10]-estratriene-3-ol (10)

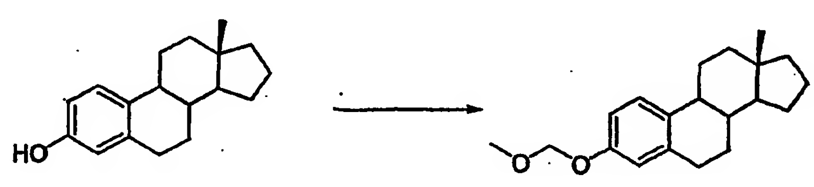


A 4M solution of methanolic HCl was prepared by cautious addition of acetyl chloride (6.1 ml) to ice cold methanol (15.6 ml), after five minutes stirring this solution was poured onto protected oestrone X (1.8 g, 4.3 mmol). Sonication was applied to speed the dissolution of the protected oestrone, a pink colour emerged in the reaction mixture after five minutes and, after a further ten minutes, ethyl acetate (100 ml), and then sufficient sodium hydrogen carbonate to neutralise the reaction mixture was added. The organic layer was then washed with water (2 x 50 ml) and brine (100 ml) before drying ($MgSO_4$) and evaporating. Purification by crystallisation (ethanol) gave a yellow crystalline solid mp. 139-141°C which showed: δ_H (400 MHz, $CDCl_3$)

7.36 (1H, s, OH), 6.73 (1H, s, ArH), 6.57 (1H, s, ArH), 2.82-91 (2H, m, 6-CH_2), 2.66 (2H, q, J 7.4, SCH_2), 1.36-2.55 (13H, m), 1.21 (3H, t, J 7.4, SCH_2CH_3) and 0.91 (3H, s, 18-CH_3); δ_C 220.5 (CO), 154.7, 140.0 (both C), 133.0 (CH), 132.2 (C), 115.7 (CH), 114.3 (C), 50.4 (CH), 48.0 (C), 43.9 (CH), 38.2 (CH), 35.9, 31.6, 31.1, 29.5, 26.5, 26.0, 21.7 (all CH_2), 15.2 and 14.0 (both CH_3); MS[FAB$^+$] 330.2 (100%, M$^+$), HRMS[FAB$^+$] 330.165352, calculated 330.16535; Calculated C, 72.69, H, 7.93; Found C 72.9 %, H, 7.99 %.

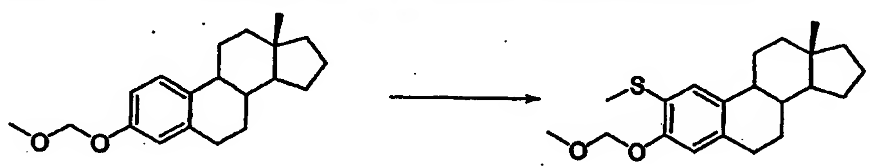
Synthesis of 2-Methylsulfanyl-17-deoxy-1,3,5[10]-estratrien-3-ol (28)

3-*O*-Methoxymethylene-17-deoxy-1,3,5[10]-estratriene



A stirred, 0°C, solution of 17-deoxy-1,3,5[10]-estratriene (3.12 g, 12.2 mmol), in dimethylformamide (50 ml) was treated sodium hydride (731 mg, 18.3 mmol) in a portion wise manner. After 0.5h stirring methyl chloromethyl ether (0.93 ml, 25 mmol) was added and the reaction mixture was left to stir overnight. Aqueous 2M ammonia (10 ml) was added to destroy excess chlorinated starting material and after a further 10 minutes ethyl acetate (200 ml) and brine (50 ml) were added. The organic layer was separated and washed five times with brine (50 ml aliquots), dried, evaporated and columned on silica gel (9:1 hexane:ethyl acetate as eluant) to give the desired protected 3-*O*-methoxymethylene-17-deoxy-1,3,5[10]-estratriene as a clear colourless oil which showed δ_H 7.21 (1H, d, *J* 8.5), 6.82 (1H, dd, *J* 8.5 and 2.3, ArH), 6.77 (1H, d, *J* 2.3, ArH), 5.14 (2H, s, OCH_2O), 3.47 (3H, s, OCH_3), 2.78-2.86 (2H, m, 6-CH_2), 1.15-2.30 (15H, m) and 0.73 (3H, s, 18-CH_3); δ_C 154.8, 138.0, 134.3, 126.3, 116.1, 113.6, 94.4, 55.9, 53.6, 44.2, 41.1, 40.6, 39.1, 38.9, 30.0, 28.2, 26.8, 25.3, 20.7 and 17.7.

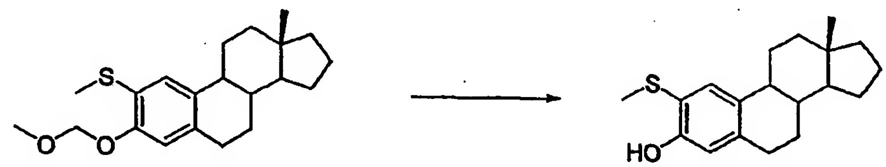
2-Methylsulfanyl-3-*O*-methoxymethylene-17-deoxy-1,3,5[10]-estratriene



A stirred solution of 3-*O*-methoxymethylene-17-deoxy-1,3,5[10]-estratriene (2.78 g, 9.35 mmol) in THF (100 ml) was cooled in a dry ice/acetone bath to –78°C before treating with *sec*-butyl lithium (28 ml, 21.5 mmol, 1.3 M solution in cyclohexane) in a dropwise manner over a period of 0.5h. The anion maintained at this temperature for 1 h and was then quenched with dimethyl disulfide (4.2 ml, 47 mmol) over a period of five minutes and then allowed to warm to room temperature over a further 2h. At this stage saturated ammonium chloride solution (10 ml) was added, the quenched solution diluted in diethyl ether (100 ml) and the organic layers were washed with saturated sodium hydrogen carbonate solution (3 x 50 ml), water (50 ml) and finally brine (50 ml) before drying ($MgSO_4$) and evaporating to yield the desired product, 2-methylsulfanyl-3-*O*-methoxymethylene-17-deoxy-1,3,5[10]-estratriene as a spectro-scopically pure yellow oil

(2.2 g, 68 %)which showed δ_H 7.16 (1H, s, ArH), 6.81 (1H, s, ArH), 5.20 (2H, s, OCH_2O), 3.51 (3H, s, OMe), 2.78-2.86 (2H, m, 6-CH_2), 2.43 (3H, s, SMe), 1.10-2.36 (15H, m) and 0.74 (3H, s, 18CH_3).

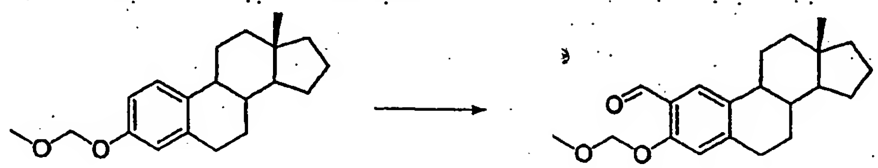
2-Methylsulfanyl-17-deoxy-1,3,5[10]-estratrien-3-ol (28)



A 4M solution of methanolic HCl was prepared by cautious addition of acetyl chloride (6.1 ml) to ice cold methanol (15.6 ml), after five minutes stirring this solution was poured onto 2-methylsulfanyl-3-*O*-methoxymethylene-17-deoxy-1,3,5[10]-estratriene (1.8 g, 5.2 mmol). Sonication was applied to speed the dissolution of the protected estrone, a pink colour emerged in the reaction mixture after five minutes and, after a further ten minutes, ethyl acetate (100 ml), and then sufficient sodium hydrogen carbonate to neutralise the reaction mixture was added. The organic layer was then washed with water (2 x 50 ml) and brine (100 ml) before drying ($MgSO_4$) and evaporating. Purification by column chromatography (8:1 hexane:ethyl acetate) gave the desired product, 2-methylsulfanyl-17-deoxy-1,3,5[10]-estratrien-3-ol, as a clear colourless oil (1.13 g, 72 %) which showed δ_H 7.41 (1H, s, OH), 6.78 (1H, s, ArH), 6.45 (1H, s, ArH), 2.78-2.85 (2H, m, 6-CH2), 1.08-2.32 (18H, m [including 2.29 (3H, s, SMe)]) and 0.74 (3H, s, 18-CH_3); δ_C 153.7, 140.2, 133.6 (all C), 131.9 (CH), 117.4 (C), 114.4, 53.5, 43.9 (all CH), 41.0 (CH_2), 40.5, 39.0 (both CH), 38.8, 29.8, 28.0, 26.9, 25.3, 20.7 (all CH_2), 20.4 and 17.6 (both CH_3); m/z [FAB+] 302.2 (M^+ + H, 100 %]; Accurate mass found 302.17044, $C_{19}H_{26}SO$ requires 302.170437.

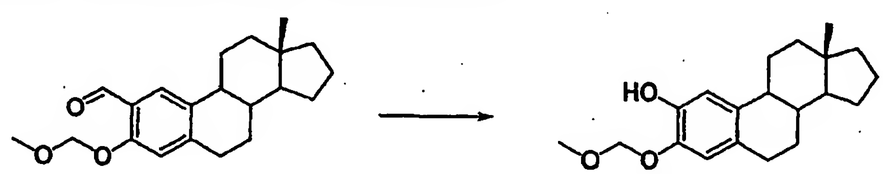
Synthesis of 2-Methoxy-17-deoxyestrone (29)

2-Formyl-3-*O*-methoxymethyl-17-deoxyestrone



A solution of 3-*O*-methoxymethylene-17-deoxy-1,3,5[10]-estratriene (2.5g, 8.33 mmol) in THF (150 ml) was cooled in a dry ice acetone bath to –75°C and then treated with sec-butyl lithium (22 ml of a 1.3M soln in hexanes, 29 mmol) over a period of 20 minutes, after a further two hours stirring freshly distilled DMF (2.5 ml, 60mmol) was added to the reaction mixture and the reaction was allowed to come to room temperature over the next 16 hours. The reaction mixture was then cooled in an ice bath before quenching with ammonium chloride solution (10 ml), adding ethyl acetate (200 ml) and transferring into a separating funnel. The organic layers were combined and washed with water (3 x 100 ml) and brine (100 ml) before drying and evaporating. The crude product was purified by column chromatography to give the product as a clear colourless oil (1.9g, 70 %) which showed δ_H 10.43 (1H, s, CHO), 7.78, (1H, s, ArH), 6.91 (1H, s, ArH), 5.26 (2H, s, OCH_2O), 3.51 (3H, s, OMe), 2.86-2.95 (2H, m, 6-CH_2), 1.10-2.41 (15H, m) and 0.73 (3H, s, 18-CH_3); δ_C 189.4, 157.2, 146.3, 134.8 (C), 125.2 (CH), 123.2 (C), 114.8 (CH), 94.5 (CH_2), 56.4 (CH_3), 53.6, 43.9 (both CH), 41.1 (C), 40.5 (CH_2), 38.9 (CH), 30.7, 27.8, 26.7, 25.3, 20.7 (all CH_2) and 17.6 (CH_3); m/z [FAB$^+$] 329.3 (M^+ + H, 80 %). Accurate mass [FAB$^+$] found 328.20385, $C_{21}H_{28}O_3$ requires 328.20385.

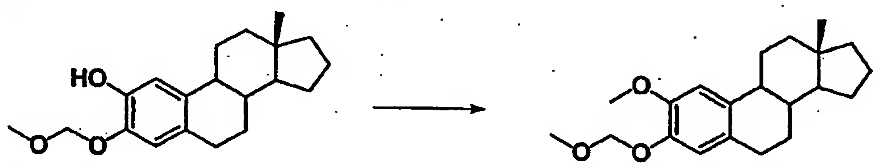
2-Hydroxy-3-*O*-methoxymethyl-17-deoxyestrone



A stirred solution of 2-formyl-3-*O*-methoxymethyl-17-deoxyestrone (1.66g, 5 mmol) in a 3:1 mixture of chloroform and dichloromethane (40 ml) was treated with dibasic sodium hydrogen phosphate (2.48g, 17.5 mmol) and then, in a dropwise manner, mCPBA (1.73 g,

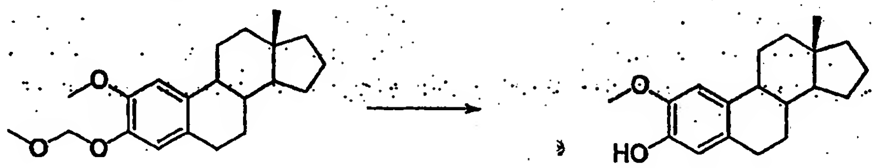
9 mmol). After five hours the reaction mixture was pured onto ice water (50 ml), extracted with dichloromethane (50 ml) and the combined organics washed with sodium hydrogen carbonate (50 ml), water (50 ml) and brine (50 ml) before drying and evaporating to give a pale yellow foam. The foam was dissolved in degassed methanol (25 ml), then treated with sodium hydroxide (9 ml, 1.8 eq) and stirred for one hour before neutralising with hydrochloric acid (1M) and removing the solvent in vacuo. The residues were diluted in ethyl acetate (40 ml) and water (40 ml), the organic layer separated, dried and evaporated to yield the crude product. Purification by column chromatography afforded the desired phenol as a clear colourless oil (1.1g, 70 %)which showed δ_H 6.90 (1H, s, ArH), 6.78 (1H, s, ArH), 5.74 (1H, s, OH), 5.15 (2H, s, OCH_2O), 3.51 (3H, s, OMe), 2.70-2.84 (2H, m, 6-CH_2), 1.08-2.26 (15H, m) and 0.73 (3H, s, 18-CH_3); δ_C 143.8, 142.1, 135.5, 128.4 (all C), 115.7, 112.3 (both CH), 96.0 (CH_2), 56.4 (CH_3), 53.6, 44.3 (both CH), 41.1 (C), 40.6 (CH_2), 39.1 (CH), 38.9, 29.3, 28.4, 26.8, 25.3, 20.7 (all CH_2) and 17.6 (CH_3).

2-Methoxy-3-*O*-methoxymethyl-17-deoxyestrone



A solution of 2-hydroxy-3-*O*-methoxymethyl-17-deoxyestrone (900 mg, 2.84 mmol) in DMF (50 ml) was treated with potassium carbonate (4.14 g, mmol) and then, after ten minutes stirring with methyl iodide (2 ml, 33 mmol) and tetrabutylammonium iodide (100 mg). After stirring for 48 h the reaction was poured onto brine (75 ml) and extracted with ethyl acetate (200 ml), the organic layer was extracted with brine (6 x 100 ml), dried and evaporated. The desired product, a clear colourless oil (300 mg, 32 %) was isolated by column chromatography and showed δ_H 6.87 (1H, s, ArH), 6.86 (1H, s, ArH), 5.19 (2H, s, OCH_2O), 3.85 (3H, s, OMe), 3.51 (3H, s, OMe), 2.75-2.86 (2H, m, 6-CH_2), 1.10-2.30 (15H, m) and 0.74 (3H, s, 18-CH_3); δ_C 144.5, 144.2, 134.5, 129.0 (all C), 116.8, 109.5 (both CH), 95.6 (OCH_2O), 56.1, 56.1 (both OCH_3), 53.6, 44.5 (both CH), 41.1 (C), 40.6 (CH_2), 39.1 (CH), 38.9, 29.3, 28.3, 27.0, 25.3, 20.7 (all CH_2) and 17.7 (CH_3).

2-Methoxy-17-deoxyestrone



A solution of methanolic HCl was formed by cautious addition of acetyl chloride (0.85 ml) to vigorously stirred ice cold methanol (2.2 ml), after ten minutes stirring the methanolic HCl was added to 2-methoxy-3-*O*-methoxymethyl-17-deoxyestrone e (200 mg, 0.61 mmol). To aid solvation of the starting material the reaction was sonicated for 5 minutes after which time saturated sodium hydrogen carbonate solution (35 ml) and then ethyl acetate was added, the organic layer was separated, the aqueous layer extracted with a further aliquot of ethyl acetate (30 ml) and the combined organic layers were washed with $NaHCO_3$ (25 ml), water (50 ml) and brine (50 ml). The resultant solution was dried and evaporated to give the crude product a yellow oil which was purified by column chromatography (chloroform) to give 2-methoxy-17-deoxyestrone as a white crystalline solid (150 mg, 86 %) mp 112-115°C. m/z (FAB^+) 286.2 (100%, M^+). Accurate mass [FAB^+] found 286.19328 $C_{19}H_{26}O_2$ requires 286.19328.

Superoxide Dismutase Assay

SOD activity was measured in accordance with Protocol 1.

Results and Discussion

We determined the SOD-inhibitory action of 27 compounds. Initial analyses were performed using test compounds at 100 μM. Compounds which gave >50% inhibition at this concentration were tested in additional titration experiments to determine IC50 values (Table).

Compound	IC50/μM (+/- SEM) SOD Inhibition
2-methylsulfanylestradiol 1	10 +/- 2
2-ethoxyestradiol 2	11 +/- 1
2-methoxy-17β-estradiol 3	12 +/- 4

Compound	IC50/μM (+/- SEM) SOD Inhibition
2-methoxy-17α-estradiol **4**	13 +/- 1
2-methoxyestrone **5**	14 +/- 2
2-ethylsulfanylestradiol **6**	14 +/-1
2-ethoxyestrone **7**	19 +/- 3
2-methylsulfanylestrone **8**	21 +/- 5
2-methoxy-3-aminoestrone **9**	79 +/- 13
2-ethylsulfanylestrone **10**	79 +/- 5
2-fluoroestrone **11**	81 +/- 1
2-chloroestrone **12**	83 +/- 1
2-methoxy-17α-benzyl-estradiol **13**	88 +/- 1
3-Hydroxy-2-methoxy-6-oxo-8,9,10,11-tetrahydro-7*H*-cyclohepta-[c][1]benzopyran **14**	91 +/- 11
2-allylestrone **15**	>100
2-iodoestrone **16**	>100
2-difluoromethylestrone **17**	>100
2-methylsulfoxyestrone **18**	>100
2-methylsulfonylestrone **19**	>100
2-nitroestrone **20**	>100
2-ethylestrone **21**	>100
2-methoxy-4-nitroestrone **22**	>100
2-methoxy-17α-(4-tert-butylbenzyl)oestradiol **23**	>100
estrone-3-O-sulphamate **24**	>100
2-methoxyestrone-3-O-sulphamate **25**	>100
2-ethylestrone-3-O-sulphamate **26**	>100
equilin-3-O-sulphamate **27**	>100
2-methylsulfanyl-17-deoxyoestrone **28**	>100
2-methoxy-17-deoxyoestrone **29**	>100

The structure-activity relationships for the potent inhibition of SOD activity by the compounds studied in this work are summarised in Figure 2.

For the 2-substituted oestrones or oestradiols that were tested here, only those compounds with either an O or S substituent show potent or good inhibitory activities against SOD. The poor inhibition exhibited by 2-ethylestrone, **21** confirms such generalisation that a heteroatom of either oxygen or sulphur at the 2-position is important for potency. Although the 2-alkoxy derivative of oestrone was a more potent inhibitor than the 2-alkylsulfanyl derivative (**7** vs **10**) the overall difference in potencies between the corresponding 2-alkoxy and 2-alkylsulfanyl pairs (**2** vs **6**, **5** vs **8**, and **1** vs **3**) was often relatively small suggesting that the enzyme tolerates the sulphur atom reasonably well despite its bigger size and lower electronegativity than oxygen atom All 2-alkylsulfanyl derivatives of oestrone or oestradiol are less potent than the corresponding 2-alkoxy derivatives (e.g. **2** vs **6**, **5** vs **8** and **7** vs **10**) with the exception of 2-methylsulfanylestradiol, **1** which appears to be the best SOD inhibitor tested here, even

better than 2-methoxyestradiol, **3**. However, the difference in potencies between the corresponding pairs (except **7** vs **10**) is relatively small which suggests that the enzyme tolerates the sulphur atom reasonably well despite its bigger size and lower electronegativity than oxygen atom.

The oxidation state of the sulphur atom is important for high potency since the tricoordinate 2-methylsulfoxy derivative, **18** and the tetracoordinate 2-methylsulphonyl derivative of oestrone, **19** are much weaker inhibitors than all divalent thio derivatives in this series such as **1** and **6**. Our data have also shown that in general the potency of a 2-alkoxy or 2-alkylsulfanyl derivative decreases with an increase in the carbon chain length of its alkyl group. It is therefore anticipated that derivatives of oestrone or oestradiol with bulkier substituents such as propoxy or propylsulfanyl at the 2-position will not be well tolerated by SOD and hence would be weaker inhibitors.

Other substituents at the 2-position are clearly detrimental as shown by the poor inhibitions of 2-allyl-, **15**, 2-difluoromethyl-, **17** and 2-nitroestrone, **20**. Interestingly, a single halo atom at the 2-position exhibits weak activity although this appears to be related to the size and presumably the electronegativity of the halogen with 2-fluoroestrone, **11** the most active and 2-iodoestrone, **16** the weakest in this series.

Replacement of the hydroxy group at the 3-position by a sulphamate group is detrimental to the inhibitory activities exhibited by their parental compounds. Whilst 2-methoxyestrone, **5** is highly potent, the IC50 for its sulphamate derivative was found to be >100 µM. However, when the hydroxy group of **5** is replaced with an amino group, compound **9** has retained a small level of inhibitory activity suggesting that the hydroxy group at the 3-position is not be an absolute requirement for activity. Clearly, any potential hydrogen bonding interaction of the 3-OH of 2-methoxyestradiol with amino acid residues in the enzyme active site could be maintained in **9**, but not in **25**.

Substitution at the 4-position of 2-methoxyestrone with a nitro group induced a significant loss in inhibitory activity of **5**. However, it is not clear if such an effect is the result of steric hindrance or electronic factors.

It was interesting that a non-steroidal tricyclic coumarin-based compound shows detectable inhibitory activity showing that the steroidal scaffold is not be an absolute

requirement for inhibitory activity. Mimicking the A-ring of 2-methoxyestradiol alone might produce inhibitory effects. To the best of our knowledge, this is the first report that a non-steroidal compound can work as a SOD inhibitor. It is anticipated that the inhibitory activity of the coumaric **14** could be further optimized to produce more potent analogues that might emerge as a new class of SOD inhibitors.

The potency of 2-methoxyestradiol is not significantly affected by the stereochemistry of the hydroxy group at the 17-position as shown by the similar potency observed for the 17β-OH derivative, **3** and its epimeric 17α-OH derivative, **4**. It does however appear that all oestradiol derivatives are better SOD inhibitors than their oestrone congeners as shown by the relative inhibitory activities of **1** vs **8**, **3** vs **5** and **2** vs **7**; this is clearest for the 2-ethylsulfanyl derivatives (**6** vs **10**). It is clear that the enzyme tolerates the 17α-benzyl group of **13** much better than the bulkier 17β-4-tert-butylbenzyl group of **23**. Hence, small substituents and structural changes at this position might not alter the potency of the parental compound to a large extent but it is likely that increasing bulky group is not tolerated.

Interestingly, the potency of the 17-deoxy analogues (**24** and **25**) proved to be very weak (IC_{50} > 100 μM). This contrasts with their parent oestradiols (**1** and **3**) and oestrones (**8** and **5**) respectively, all of which show potent inhibitory activity. Hence, deletion of these 17 position motifs leads to a dramatic loss of activity. The similar activity of both parent oestradiols (potential H-bond acceptors and donors at C-17) and oestrones (potential H-bond acceptors at C-17) imply that the presence of an H-bond acceptor at the 17-position could be important in binding to SOD and deletion of this motif in **24** and **25** abolishes activity. Notably the 17-α derivative **4** is still potent implying that its O-atom of the 17-OH can still potentially act as an H-bond acceptor.

Conclusions

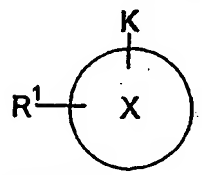
Our study has shown that synthetic analogues can be equipotent to, or more potent than, 2-methoxyestradiol, a natural ligand for the SOD enzyme. A S or O containing substituent is pivotal for SOD inhibition. We demonstrate here, for the first time, that the steroidal skeleton of 2-methoxyestradiol can be replaced by a non-steroidal scaffold without a complete loss of inhibitory activity. Such observations, together with the structure-activity relationships for SOD inhibition derived from this work, will facilitate the

design of more potent steroidal and non-steroidal-based SOD inhibitors in the future.

All publications and patents and patent applications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry, biology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. Use of a compound in the manufacture of a medicament to inhibit superoxide dismutase (SOD) or for use in the therapy of a condition or disease associated with SOD wherein the compound is of Formula I



Formula I

wherein:

X is a ring having at least 4 atoms in the ring;

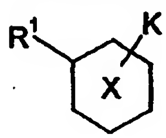
K is a hydrocarbyl group;

R^1 is a halogen or a group of the formula $-L^1-Z-R^{1'}$, wherein L^1 is an optional linker group, Z is O or S and $R^{1'}$ is a hydrocarbyl group or H;

with the proviso that the compound is other than 2-methoxy-17β-oestradiol, 2-methoxyoestrone and 2-hydroxyestradiol.

2. Use according to claim 1 wherein X is a 6 membered ring.

3. Use according to claim 1 or 2 having the Formula II



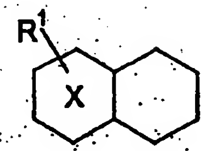
Formula II

4. Use according to any one of the preceding claims wherein K is a cyclic group.

5. Use according to any one of the preceding claims wherein the compound is a polycyclic compound.

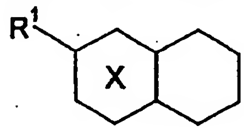
6. Use according to any one of the preceding claims wherein X in combination with K is a polycyclic ring structure.

7. Use according to any one of the preceding claims having the Formula III



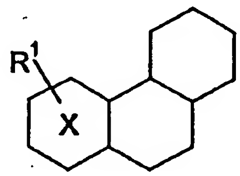
Formula III

8. Use according to any one of the preceding claims having the Formula IV



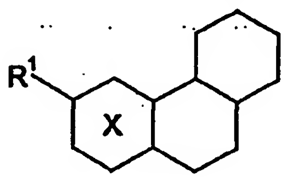
Formula IV

9. Use according to any one of the preceding claims having the Formula V



Formula V

10. Use according to any one of the preceding claims having the Formula VI

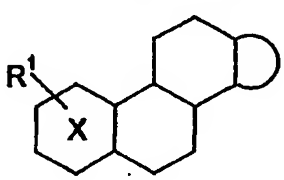


Formula VI

11. Use according to any one of the preceding claims wherein X in combination with K is a steroidal ring structure.

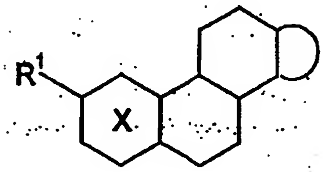
12. Use according to any one of the preceding claims wherein the compound X in combination with K mimics a steroidal structure.

13. Use according to any one of the preceding claims having Formula VII



Formula VII

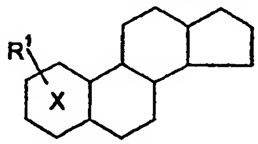
14. Use according to any one of the preceding claims having Formula VIII



Formula VIII

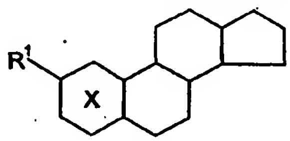
15. Use according to any one of the preceding claims having Formula IX

Formula IX



16. Use according to any one of the preceding claims having Formula X

Formula X



17. Use according to any one of the preceding claims wherein R^1 is a halogen.

18. Use according to claim 17 wherein the halogen is selected from F and Cl.

19. Use according to any one of claims 1 to 16 wherein R^1 is a group of the formula $-L^1-Z-R^{1'}$.

20. Use according to claim 19 wherein R^1 is a group of the formula $-Z-R^{1'}$.

20. Use according to claim 19 or 20 wherein Z is O.

21. Use according to claim 19 or 20 wherein Z is S.

22. Use according to any one claims 19 to 21 wherein $R^{1'}$ is an alkyl group.

23. Use according to any one of claims 19 to 22 wherein $R^{1'}$ is a C_1-C_{10} alkyl group.

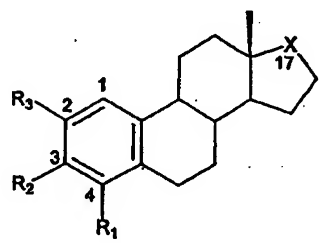
24. Use according to any one of claims 19 to 23 wherein $R^{1'}$ is a C_1-C_6 alkyl group.

25. Use according to any one of claims 19 to 24 wherein $R^{1'}$ is a C_1-C_3 alkyl group.

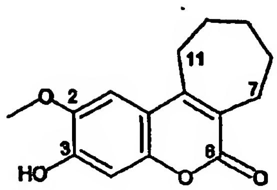
26. Use according to any one of claims 19 to 25 wherein $R^{1'}$ is $-CH_3$ or $-CH_2CH_3$.

27. Use according to any one of claims 1 to 16 wherein R^1 is selected from $-SCH_3$, OCH_3, $-SCH_2CH_3$, $-OCH_2CH_3$, $-F$, and $-Cl$.

28. Use according to claim 1 wherein the compound is selected from compounds of the formulae



R_1	R_2	R_3	X	
H	OH	MeS	CH(β-OH)	1
H	OH	EtO	CH(β-OH)	2
H	OH	MeO	CH(α-OH)	4
H	OH	EtS	CH(β-OH)	6
H	OH	EtO	C=O	7
H	OH	MeS	C=O	8
H	NH_2	MeO	C=O	9
H	OH	EtS	C=O	10
H	OH	F	C=O	11
H	OH	Cl	C=O	12
H	OH	MeO	C(β-OH)(α-Bn)	13
H	OH	$H_2C{=}CHCH_2$	C=O	15
H	OH	I	C=O	16
H	OH	CHF_2	C=O	17
H	OH	MeSO	C=O	18
H	OH	$MeSO_2$	C=O	19
H	OH	NO_2	C=O	20
H	OH	Et	C=O	21
NO_2	OH	MeO	C=O	22
H	OH	MeO	C[β-OH][α-(4-^{t}Bu)Bn]	23
H	OSO_2NH_2	H	C=O	24
H	OSO_2NH_2	MeO	C=O	25
H	OSO_2NH_2	Et	C=O	26



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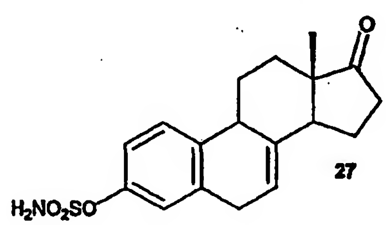

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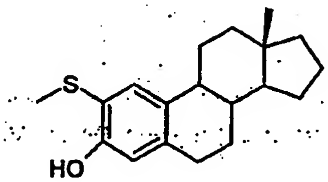

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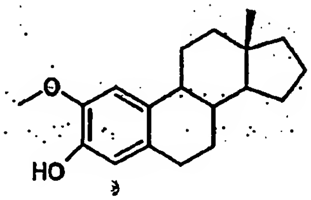

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29. Use according to claim 1 wherein, when R1 is a methoxy group or hydroxy group, X in combination with K is a non-steroidal ring structure.

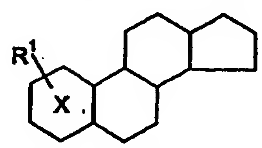
30. Use according to claim 1 wherein the compound is non-steroidal.

31. Use according to claim 1 wherein X in combination with K is a non-steroidal ring structure.

32. Use according to claim 1 wherein $R^{1'}$ is a C_2-C_{10} alkyl group, preferably a C_2-C_8 alkyl group, preferably a C_2 or C_3 alkyl group.

33. Use according to claim 1 wherein when Z is O, $R^{1'}$ is a C_2-C_{10} alkyl group; and when Z is S, $R^{1'}$ is a hydrocarbyl group or H

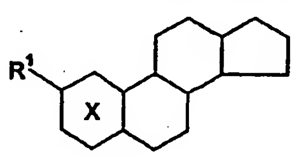
34. A compound of having Formula IX



Formula IX

wherein R^1 is a group of the formula $-L^1$-S-$R^{1'}$, wherein L^1 is an optional linker group, and $R^{1'}$ is a hydrocarbyl group or H.

35. A compound of having Formula X



Formula X

wherein R^1 is a group of the formula $-L^1$-S-$R^{1'}$, wherein L^1 is an optional linker group, and $R^{1'}$ is a hydrocarbyl group or H.

36. A compound according to claim 34 or 35 wherein R^1 is a group of the formula –S-$R^{1'}$.

37. A compound according to claim 34, 35 or 36 wherein $R^{1'}$ is an alkyl group.

38. A compound according to claim 37 wherein $R^{1'}$ is a C_1-C_{10} alkyl group.

39. A compound according to claim 37 wherein $R^{1'}$ is a C_1-C_6 alkyl group.

40. A compound according to claim 37 wherein $R^{1'}$ is a C_1-C_3 alkyl group.

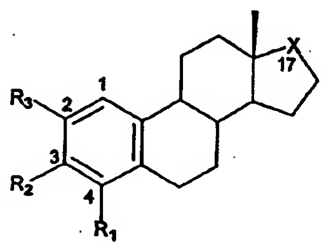
41. A compound according to claim 37 wherein $R^{1'}$ is $-CH_3$ or $-CH_2CH_3$.

42. A compound according to claim 34 or 35 wherein R^1 is selected from $-SCH_3$, $-SCH_2CH_3$, $-S(O)CH_3$, and $-S(O)(O)CH_3$

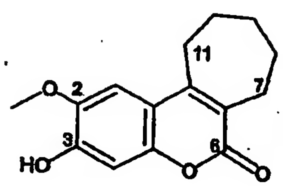
43. A compound according to any one of claims 34 to 42 for use in medicine.

44 A pharmaceutical composition comprising the compound according to any one of claims 34 to 42 optionally admixed with a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

45. Use of a compound according to any one of claims 34 to 42 in the manufacture of a medicament for the inhibition of SOD or for use in the therapy of a condition or disease associated with SOD.



R1	R2	R3	X	
H	OH	MeS	CH(β-OH)	1
H	OH	EtO	CH(β-OH)	2
H	OH	MeO	CH(β-OH)	3
H	OH	MeO	CH(α-OH)	4
H	OH	MeO	C=O	5
H	OH	EtS	CH(β-OH)	6
H	OH	EtO	C=O	7
H	OH	MeS	C=O	8
H	NH_2	MeO	C=O	9
H	OH	EtS	C=O	10
H	OH	F	C=O	11
H	OH	Cl	C=O	12
H	OH	MeO	C(β-OH)(α-Bn)	13
H	OH	$H_2C=CHCH_2$	C=O	15
H	OH	I	C=O	16
H	OH	CHF_2	C=O	17
H	OH	MeSO	C=O	18
H	OH	$MeSO_2$	C=O	19
H	OH	NO_2	C=O	20
H	OH	Et	C=O	21
NO_2	OH	MeO	C=O	22
H	OH	MeO	C[β-OH][α-(4-^{t}Bu)Bn]	23
H	OSO_2NH_2	H	C=O	24
H	OSO_2NH_2	MeO	C=O	25
H	OSO_2NH_2	Et	C=O	26



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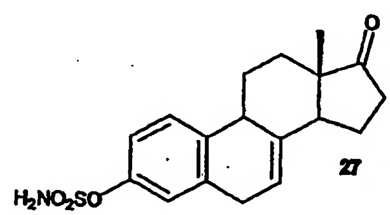

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Figure 1 Structures of compounds 1 to 27

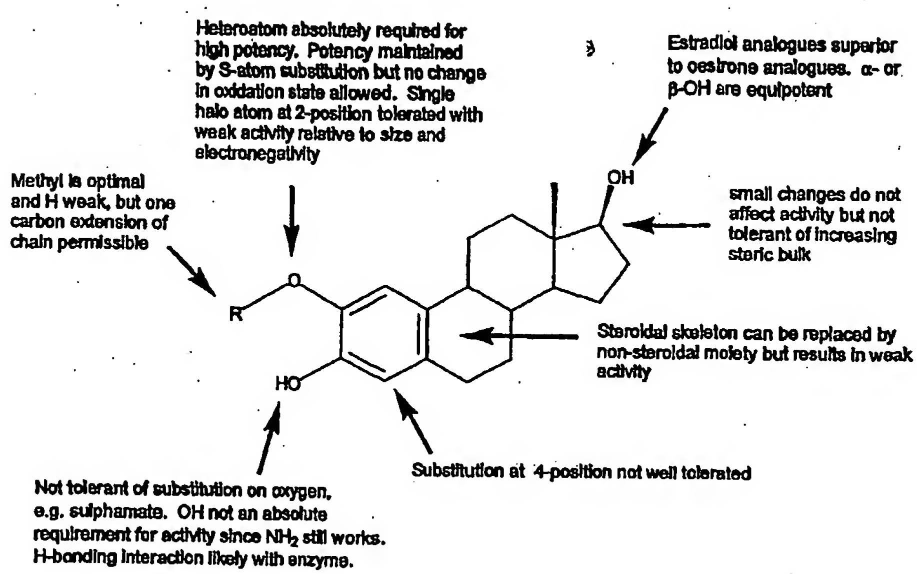

Figure 2. Summary of the structure-activity relationships for SOD inhibition for the compounds studied.

INTERNATIONAL SEARCH REPORT

Inte[…]nal Application No
PCT/GB 01/03715

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/565 A61K31/37 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P C07J C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CUSHMAN M ET AL: "SYNTHESIS, ANTITUBULIN AND ANTOMITOTIC ACTIVITY, AND CYTOTOXICITY OF ANALOGS OF 2-METHOXYESTRADIOL, AN ENDOGENOUS MAMMALIAN METALBOLIC OF ESTRADIOL THAT INHIBITS TUBULIN POLYMERIZATION BY BINDING TO THE COLCHICINE BINDING SITE" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 38, no. 12, 9 June 1995 (1995-06-09), pages 2041-2049, XP002059349 ISSN: 0022-2623 page 2042 -page 2043; table 1	28

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2001	16/01/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Steendijk, M

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/03715

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CUSHMAN ET AL.: "Synthesis of Analogs..." J. MED. CHEM., vol. 40, 1997, pages 2323-2334, XP002186112 page 2323 -page 2327; table 1	28
X	SCHNEIDER ET AL.: "Antiestrogen action of 2-hydroxyestrone.." J. BIOL. CHEM., vol. 259, no. 8, 1984, pages 4840-4845, XP002186113 abstract	28
X	WO 99 64013 A (POTTER BARRY VICTOR LLOYD ;REED MICHAEL JOHN (GB); STERIX LTD (GB)) 16 December 1999 (1999-12-16) page 35	28
X	EP 0 240 717 A (UNIV NEBRASKA) 14 October 1987 (1987-10-14) page 17; claim 1	28
A	WO 97 30041 A (UNIV BATH ;REED MICHAEL J (GB); IMPERIAL COLLEGE (GB); POTTER BARR) 21 August 1997 (1997-08-21) claim 1	28
Y	RAJAN R ET AL: "ESTROGEN EFFECTS ON NADH OXIDASE AND SUPEROXIDE DISMUTASE IN PREPUBERTAL FEMALE RATS" STEROIDS, ELSEVIER SCIENCE PUBLISHERS, NEW YORK, NY, US, vol. 40, no. 6, 1 December 1982 (1982-12-01), pages 651-660, XP002059348 ISSN: 0039-128X page 656	28
Y	KONG Q ET AL: "ANTIOXIDANT INHIBITION-BASED STRATEGY FOR CANCER THERAPY" PROCEEDINGS OF THE 90TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. PHILADELPHIA, PA, APRIL 10 - 14, 1999, PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, PHILADELPHIA, PA: AACR, US, vol. 40, March 1999 (1999-03), page 53ABSTRACT XP000882941 the whole document	28

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	HUANG ET AL.: "Superoxide dismutase.." NATURE, vol. 407, 21 September 2000 (2000-09-21), page 390-395 XP002186114 cited in the application the whole document	28
T	KACHADOURIAN ET AL.: "2-Methoxyestradiol does not inhibit..." ARCH. BIOCHEM. BIOPHYS., vol. 392, no. 2, 15 August 2001 (2001-08-15), pages 349-353, XP002186115	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-45

The claims and the description are inconsistent to the extend that a meaningfull search can only be carried out for a limited part of the claimed subject-matter. In particular, the claims define the use of a wildly broad group of substituted cyclic compounds (compare claims 1-33) as well as a broad sub-group of compounds per se (steriods carrying at the A-ring a sulfur comprising substitution), whereas the description (see table, pages 53-54) indicates that the relevant activity (SOD-inhibition) would only be observed in a very limited set of examples (compounds 1-14), with this activity appearing very sensitive to various modifications (see in-active examples 15-29, see also fig. 2/2).
The search has therefore been limited to the relevant use of compounds of claims 28, which appear active in the table on page 53-54, and to the active compoudns comprising a sulfur-substitution per se.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.